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KEY FACTORS INFLUENCING THE OCCURRENCE AND FREQUENCY OF CIGUATERA

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Statement of the contributions of others

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The table below documents the contribution and extent of intellectual input of each author, including the candidate (Sparrow, Leanne), shown in bold face.

Chapter No.	Details of publication on which chapter is based	Nature and extent of the contribution of each author, including the candidate
1	Heimann, K. and **Sparrow, L.** (2015) Chapter 37: In: Kim, S.-K. [ed] *Handbook of Marine Microalgae Biotechnology Advances*. Elsevier, London, pp. 547-558	Both authors contributed ***equally*** to the development of the book chapter, including data collecting, draft and editoral as well as preparation of figures and tables.
2	**Sparrow, L.,** Heimann, K., Bielig, L., Capper, A., and Blair, D. (*in prep*). Marine and Freshwater Research	Heimann and **Sparrow** collected the data. **Sparrow** drafted the paper which was revised with editorial input from Heimann, Bielig, Capper and Blair. **Sparrow** developed the figures and tables, except Figure which was developed by **Sparrow** and Capper.
3	**Sparrow, L.,** Heimann, K., and Connolly, S. R. (*in prep*). Harmful Algae	**Sparrow** developed the research question. Data collected by Heimann and **Sparrow**. Modelling performed by Connolly. **Sparrow** drafted the paper which was revised with editorial input from Heimann and Connolly. **Sparrow** developed figures and tables, excluding modelling tables, which were developed by Connolly.
4	**Sparrow, L.** and Heimann, K. (2016). *Journal of Coastal Research*, 75:1007-1011.	**Sparrow** and Heimann developed the research question. **Sparrow** collected field data with assistance by numerous volunteers. **Sparrow** performed data analysis, and prepared figures and tables. **Sparrow** drafted the paper with editorial input from Heimann.
5	**Sparrow, L.,** Momigliano, P., Russ, G. R., and Heimann, K. (2017). Harmful Algae 65:52-60	**Sparrow** and Heimann developed the research question. **Sparrow** collected the data and performed the data analysis with assistance from Russ and Empro. Rhondda Jones. Momigliano performed genetic analysis. **Sparrow** drafted the paper with editorial input from Momigliano, Russ and Heimann. **Sparrow** developed the figures and tables, except for phylogeny tree which was prepared by Momigliano.
6	**Sparrow, L.,** von Alvensleben, N., de Royer, A., and Heimann, K. (*submitted*). Journal of experimental marine biology and ecology	**Sparrow** and Heimann developed the research question. **Sparrow** collected the data with assistance from de Royer and von Alvensleben. **Sparrow** and Heimann performed the data analysis. **Sparrow** drafted the paper with editorial input from Heimann, von Alvensleben and de Royer. Figures and tables developed by **Sparrow** with assistance from Heimann.

Abstract

Ciguatera is a human illness caused by ingestion of toxic dinoflagellates. It is endemic to tropical regions, but has expanded globally, facilitated by increased tourism to the tropics and the distribution of frozen fish from the tropics. Fish sourced from the Great Barrier Reef (GBR) and Queensland (Qld) coastal waters are the primary source of ciguatera in Australia, however, environmental drivers of ciguatera remain poorly understood. The main causative dinoflagellate genus, *Gambierdiscus*, produce ciguatoxins that bioaccumulate through marine food webs. *Gambierdiscus* species are frequently found on macroalgal substrates and usually co-occur with other benthic dinoflagellates, such as *Prorocentrum* and *Ostreopsis*. *Gambierdiscus* species have been recorded in the GBR (17 ºS, 20 – 25 ºS, 27ºS) and very recently have been recorded as far south as Merimbula (37 ºS), New South Wales (NSW). Eco-physiological drivers for population range expansions are unknown, but increasing sea surface temperatures (SSTs) might facilitate range expansion south. Climate change scenarios predict an increase in frequency and intensity of warmer than average periods and environmental disturbances, which will impact coral reef health. The response of *Gambierdiscus* and the flow-on effect on ciguatera incidence under predicted climate change conditions are unknown for Qld. The main objective of this thesis was to contribute to the current state of knowledge on ciguatera in Qld, Australia. The focus was on the potential effect of climate, i.e. increased SSTs and environmental stressors, specifically changes in salinity, on the occurrence of ciguatera and the potential for range

expansion of *Gambierdiscus* populations southward into colder coastal marine habitats.

Warmer SSTs associated with climatic events, such as El Niño and the Pacific Decadal Oscillation (PDO) have been linked to increases in ciguatera incidence for several island nations in the South Pacific region. Coral bleaching is often triggered by warmer SSTs, while disturbances from cyclones and crown-of-thorns starfish (*Acanthaster planci*) outbreaks frequently co-occur, providing new substrates for macroalgae and associated benthic dinoflagellates to colonise. The effect of altered climate and environmental disturbances on the occurrence of ciguatera in Qld has not been explored previously. A desk study was conducted on ciguatera cases reported in an Australian government publicly accessible health database. Data were analysed and related to records of climate and environmental disturbances during the same period. Ciguatera cases were higher in the PDO warm phase than in the cool phase, and coral bleaching significantly lowered incidence of ciguatera cases in the year of bleaching. The large range of latitude on Qld east coast (10 – 28 ºS) made identification of clear links between ciguatera outbreaks and El Niño and other disturbance events difficult to identify. Thus, drivers of *Gambierdiscus* population responses to environmental changes need to be identified.

Macroalgal substrate preferences of *Gambierdiscus*, in terms of phyla and the chemical and structural defences of macroalgae against colonisation of *Gambierdiscus* remain unclear. Abundances of *Gambierdiscus* have been reported in the northern and southern regions of the GBR, but not in the central GBR. Field surveys conducted for at inshore and mid-shelf reef sites in the

central GBR investigated benthic dinoflagellate abundances and preferences of *Gambierdiscus* for different macroalgal substrates, based on thallus form and grazing pressures. There appeared to be no macroalgal substrate preference of *Gambierdiscus*, but abundance of *Gambierdiscus* varied with sites. In the absence of substrate preference and with the propensity of macroalgae to colonise substrates, benthic dinoflagellates are likely to expand populations southward into new geographic regions.

Temperature and salinity can physiologically challenge survival of benthic dinoflagellates and thus expansion of geographic range. On the GBR, inshore reefs experience more variable temperatures and salinities relative to reefs further offshore, and such variations are often influenced by environmental disturbances. In the central GBR, field surveys found that *Gambierdiscus* co-occur with high abundances of *Prorocentrum* and *Ostreopsis*, however, it remains unclear whether mixed benthic dinoflagellate assemblages have a positive effect on growth of *Gambierdiscus* populations in periods of environmental stress. Fully factorial experiments were conducted to investigate firstly, the effect of temperature and salinity on two strains of *G. carpenteri* isolated from GBR waters (NQAIF116 and NQAIF380); and secondly, the effect of salinity and the presence of a mixed benthic dinoflagellate assemblage on the population growth of the inshore *G. carpenteri* strain, NQAIF116. NQAIF116 showed a greater potential for range expansion southward into colder estuarine environments. Range expansion, however, would not lead to an increase in incidence of ciguatera unless the benthic dinoflagellates were taken up by resident fish populations, a possibility which requires further research.

It is thought that the summer occurrence of *G. carpenteri* in southern waters off Merimbula, NSW was caused by transport of the dinoflagellate by the East Australian Current (EAC), which can reach further south in a warmer climate. Temperature and salinity changes could affect the biochemical profile of *Gambierdiscus* species transported into more southern waters, potentially affecting their nutritional qualities, such as their long-chain polyunsaturated fatty acids (LC-PUFAs) which are essential components in marine food webs. Large-scale cultures of *G. carpenteri*, *Prorocentrum lima* and *Ostreopsis* sp. were set up to investigate whether temperature and salinity, which appeared to drive population growth in small-scale cultures, would affect nutritional profiles of these dinoflagellates. Culture nutrient status was kept replete for nitrate and phosphate. Growth and nutrient uptake rates of dinoflagellates were measured every second day in cultures with replete levels of nitrate and phosphate. Temperature and salinity changes had negligible effects on Total Fatty Acids (FA), Saturated Fatty Acids (SFA), Monounsaturated Fatty Acids (MUFA) and Polyunsaturated Fatty Acids (PUFA) in *G. carpenteri*, *P. lima* and *Ostreopsis* sp. This suggests that benthic dinoflagellates remain a good source of essential LC-PUFAs even in conditions of changed temperature and salinity. Population growth rate of *P. lima* (a species that has been implicated in diarrhetic shellfish poisoning) is temperature and salinity tolerant. This could have implications for oyster beds in NSW. Population growth rate of *Ostreopsis* was salinity-tolerant, but affected slightly by temperature, while population growth rate of *G. carpenteri* was temperature-tolerant, but negatively affected by lower salinity. This has implications for the potential of ciguatoxin transfer, the rate of which is

considered high in offshore waters, but relatively low in inshore and estuary waters. This suggests that a useful extension of the present research would be to investigate toxin production in dinoflagellates under temperature and salinity stress.

As predicted climate change conditions will impact on coral reef health, generating more substrate on the GBR for macroalgae and associated benthic dinoflagellate assemblages to colonise. The principle aim of research in this thesis was to better understand potential drivers of ciguatera occurrence and the effect of predicted climate change on the potential range expansion of benthic dinoflagellates into new geographic regions. This was achieved by investigating ocean-scale and decadal-scale environmental changes and environmental disturbances with respect to a unique, long-term dataset on reported ciguatera cases for Qld (chapter 3), evaluating distribution and substrate preferences of potentially harmful benthic dinoflagellates (chapter 4), investigating responses to temperature and salinity changes on population growth of dinoflagellates (chapter 5) and investigating nutritional value of benthic dinoflagellates at a lower salinity and temperature, as a novel approach to evaluate potential uptake of ciguatoxins into marine food webs (chapter 6). These findings have improved our understanding of the influence of ocean-scale climatic processes in predicting ciguatera occurrence, and data suggest a potential for southward geographic expansion of ciguatera occurrence into more southern coastal environments as climate changes.

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Chapter Two

In prep CSIRO Marine and Freshwater Research

Chapter Three

In prep Harmful Algae

Chapter Four

Sparrow, L. and Heimann, K. (2016) Key environmental factors in the management of ciguatera. *Journal of Coastal Research*, 75:1007-1011.

Chapter Five

Sparrow, L., Momigliano, P., Russ, G. R., and Heimann, K. (2017) Effects of temperature, salinity and composition of the dinoflagellate assemblage on the growth of *Gambierdiscus carpenteri* isolated from the Great Barrier Reef. Harmful Algae 65:52-60.

Chapter 6

In prep Journal of Experimental Marine Biology and Ecology

Chapter 7

In prep Harmful Algae

Chapter One: Introduction[1]

1.1 Aim and Structure of this thesis

The objective of this research was to contribute significantly to the current state of knowledge and understanding of ciguatera in Queensland, Australia. The research investigated key factors relating to climate change that influence the occurrence and frequency of ciguatera in the Great Barrier Reef. The aims of this research were to:

- Determine the present state of knowledge on ciguatera research in Queensland (Qld);
- Determine whether ciguatera incidence in Qld is linked to climatic periods and environmental disturbances;
- Determine the occurrence and likely substrate preference of ciguatera-causing benthic dinoflagellates in the central Great Barrier Reef (GBR);
- Investigate environmental tolerances of benthic dinoflagellates isolated from the GBR to determine potential for range expansion under changed climatic conditions; and

1 This chapter is adapted from
Heimann, K. and ***Sparrow, L.*** (2015) Chapter 37: Ciguatera – tropical reef fish poisoning. In: Kim, S.-K. [ed] *Handbook of Marine Microalgae Biotechnology Advances*. Elsevier, London, pp. 547-558

Both authors contributed equally to the development of the book chapter.

The chapter has been updated to reflect the current state of knowledge in the field and has been modified to fit the thesis flow.

- Investigate the effect of temperature and salinity on the nutritional profile and growth potential of benthic dinoflagellates isolated from the GBR and to infer potential for southward geographic expansion of these dinoflagellates.

Chapters 2 – 6 have been prepared for publication, with an introduction specific for each chapter. Therefore, only a brief introduction to ciguatera, its importance and major knowledge gaps are presented in chapter 1.

1.2 General Introduction

Ciguatera has been recorded in tropical regions since the 16th century (Lewis, 1986b; Rhodes et al., 2010) and is now considered the most-reported seafood-associated illness worldwide (Arena et al., 2004; Friedman et al., 2008; Stinn et al., 2000). It is induced by the consumption of tropical fish, which have accumulated ciguatoxins through their diet. Ciguatoxins are derived from *Gambierdiscus* spp, benthic toxic dinoflagellates found in assemblages with other benthic toxic dinoflagellates, most frequently *Prorocentrum* and *Ostreopsis* species. It is hypothesized that benthic toxic dinoflagellates are taken up by fish grazing on macroalgal substrates (Randall, 1958; Rongo and van Woesik, 2011). The toxins are then bio-converted to more potent forms and trophically transferred to mesopredators, such as mackerel and coral trout that are often targeted by fishing for human consumption (Heimann et al., 2011; Lewis et al., 1991; Tester et al., 2013).

1.2.1 Symptoms of ciguatera

Ciguatera produces a range of gastrointestinal, neurological and cardiovascular symptoms in humans. Diagnosis is complicated by variation in severity of illness (depending on the individual affected and the dose of toxin), timing of onset of symptoms (usually 1 to 48 hours after consuming fish), absence of a set sequence of symptoms experienced, and regional variation in symptoms (Botana, 2000). The debilitating effects of neurological symptoms can persist for short periods or can extend over months or even years. The symptoms can be retriggered by later consumption of seafood, as well as consumption of non-seafood based-foods, such as chicken, peanuts and alcohol (Donati, 2006). The most frequently reported neurological symptoms include headache, sweating, fatigue, pruritus (intense itching), paraesthesia (tingling of the oral region and/or body extremities), arthralgia (joint and muscle pain), sensation of temperature cold-hot reversal and paresis (muscular paralysis) (Donati, 2006). Nausea, vomiting, diarrhea and abdominal cramps are commonly reported gastrointestinal symptoms, while cardiovascular symptoms include low blood pressure and an erratic pulse (Donati, 2006).

Variability of ciguatera incidence in the Caribbean and Pacific regions (Dickey and Plakas, 2010) has been attributed to several factors. Geographical differences in symptoms of ciguatera might reflect the region-specific differences in toxin profiles (Lewis, 2001) of the 16 species of *Gambierdiscus* (*G. toxicus, G. pacificus, G. australes, G. polynesiensis, G. belizeanus, G. caribaeus, G. carpenteri, G. ruetzleri, G. balechii, G. lapillus, G. honu, G. carolinianus, G. excentricus, G. scabrosus, G. silvae, G. cheloniae* – see

Appendix A). Gastrointestinal symptoms are the most commonly reported symptoms in the Caribbean (Figure 1.1A). By contrast, neurological symptoms are more common in the Pacific region (Figure 1.1B). This suggests that in addition to toxin dose, the ciguatoxin type affects the symptoms of ciguatera.

1.2.2 Ciguatera incidence

Nowadays, increased affordability of international travel and a growing consumer demand for tropical reef fish in temperate and sub-tropical regions (Figure 1.2), has led to a worldwide risk of ciguatera with estimates of 500,000 people being affected annually (Arena et al., 2004; Dickey and Plakas, 2010; Donati, 2006). In tropical regions, estimated annual incidence of ciguatera can be highly variable: some island nations report more than 100 cases/ 10,000 people, while others report incidences lower than 10 cases/ 10,000 people (Lewis, 1986b; Skinner et al., 2011; Tester et al., 2010). Although an overall 60% increase of ciguatera incidence in the Pacific was recorded between 1998 and 2008, variation between Pacific islands remains (Skinner et al., 2011). Some regions, such as French Polynesia and Tokelau, have had relatively stable annual incidence of ciguatera over the years. Other regions, such as the Cook Islands, have experienced great variation in incidences: one case/100,000 for 1983-1993 to 1436/100,000 for 1998-2008 (Skinner et al., 2011). Hotspots for ciguatera (Figure 1.2) include French Polynesia, Kiribati, Vanuatu and the US Virgin Islands (Chinain et al., 2010; Skinner et al., 2011; Tester et al., 2010). In Australia, frequent local occurrences of ciguatera has led to coastal areas in the Gulf of Carpentaria, Northern Territory being recognised as local hotspots (Lucas et al., 1997), while in Queensland (Qld), Platypus Bay, Fraser Island is a

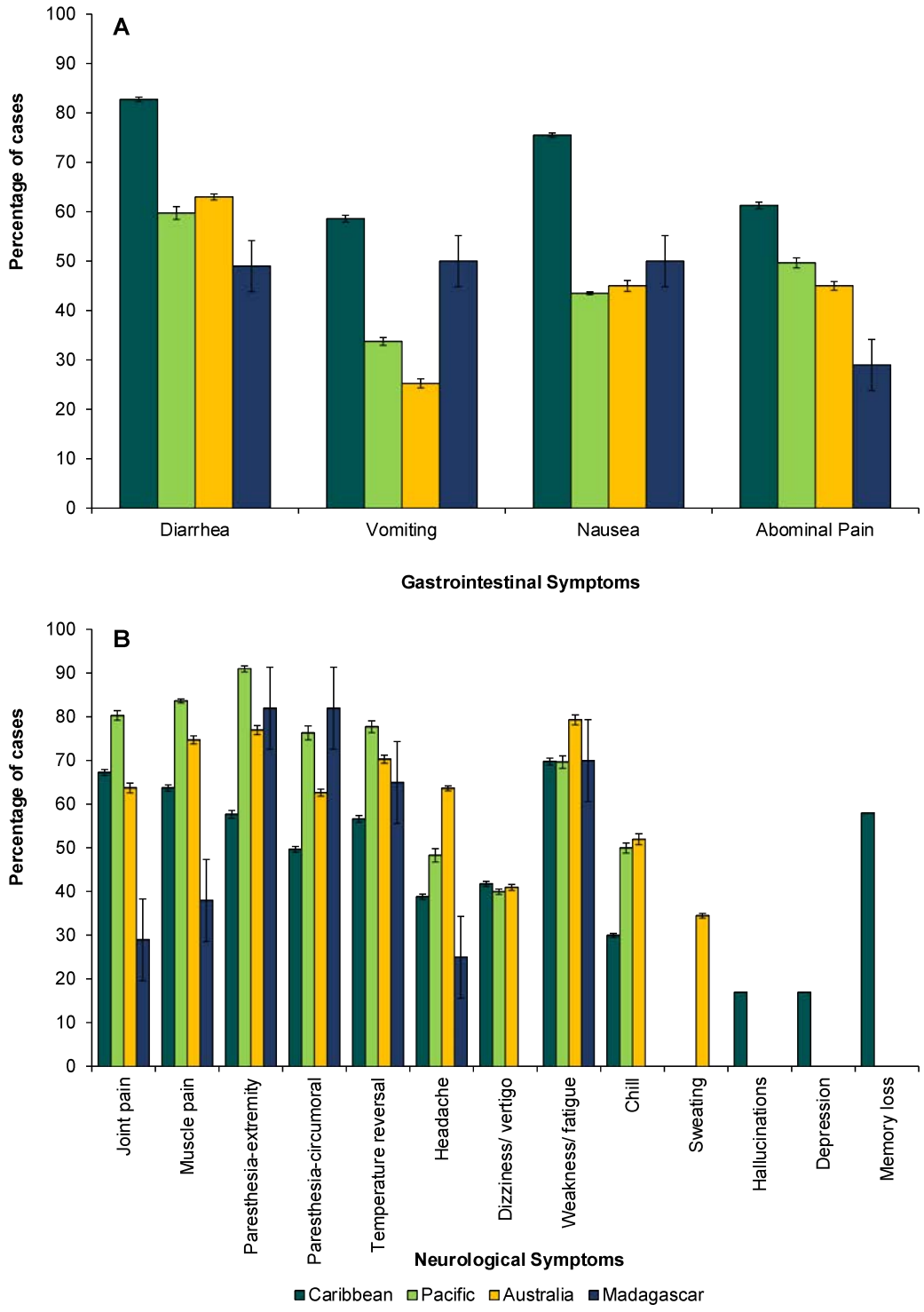

Figure 1.1 Percentage of (A) gastrointestinal symptoms and (B) neurological symptoms (mean ± SE; except Madagascar with 5% error as only a single case study) in reported ciguatera cases. (Arena et al., 2004; Barkin, 1974b; Baumann et al., 2010; Gillespie et al., 1986; Harvey, 1997; Morris et al., 1982; Neville and Warren, 2003; Ng and Gregory, 2000; Ting et al., 1998)

globally known hotspot after Spanish mackerel sourced from the area and sent to the Sydney Fish Markets was linked to an outbreak in Sydney, New South Wales (NSW) (Lehane and Lewis, 2000). In Qld, an annual incident rate of three cases / 10,000 population was estimated based on a telephone survey conducted in 1985 within Cairns, northern Qld and Maryborough, southern Qld (Capra and Cameron, 1985). At the time, this estimate was comparable with the ciguatera incidence rate in the Pacific. While recent questionnaires in the Pacific have identified that the incidence rate has increased by 66% (Skinner et al., 2011), the prevalence of ciguatera in Qld has not been reviewed recently.

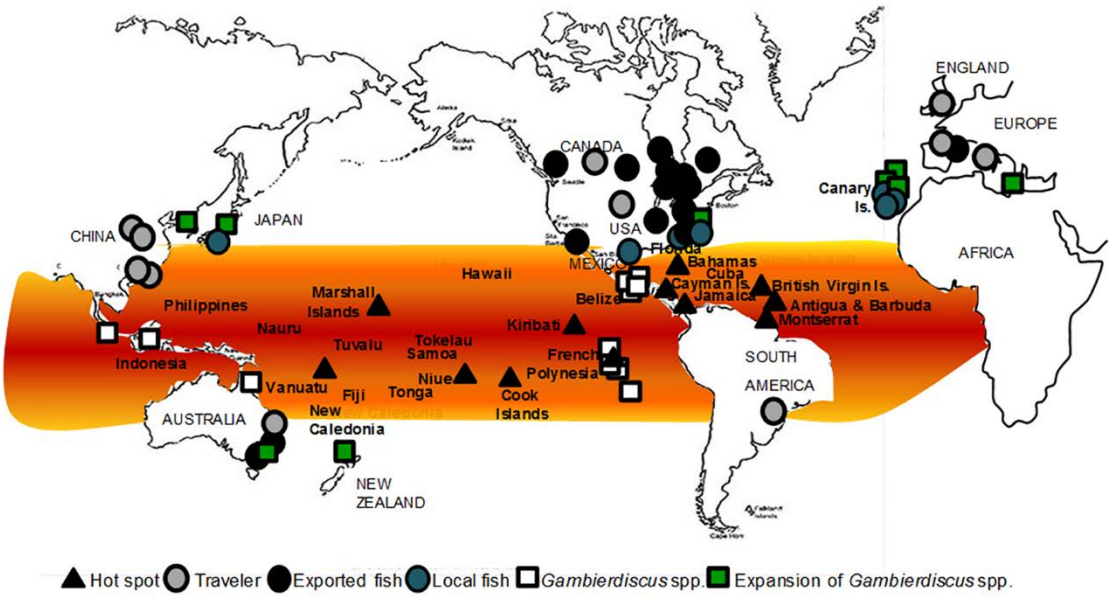

Figure 1.2 Frequency and occurrence of ciguatera hot spots (black triangles), reported ciguatera cases outside tropical regions (orange-red band) associated with travellers (grey circles) and fish exports (black circles), *Gambierdiscus* spp. isolates within tropical regions (white squares) and range expansions into temperate regions (green squares), (for additional description and references, see supplementary Table S1.1, Appendix A).

1.2.3 Economic constraints and considerations

Ciguatera management practices globally remain limited to restrictions and bans on commercial purchase or capture of commonly implicated fish species,

as preventative solutions have not yet been developed. These solutions could include an affordable and reliable test kit for detection of ciguatoxins in captured fish and monitoring systems for ciguatera-causing dinoflagellates. Existing management practices have limited the development of local marine fisheries on Caribbean and Pacific islands dependent on the export trade (Anderson and Lobel, 1987; Olsen et al., 1984). Economic losses induced by ciguatera can impact at national, regional and local levels through a) the loss of faith in the primary industry and/or export restriction for certain fish species, b) effects on tourism in known ciguatera hot spots, and c) reduced income (Bagnis et al., 1990; Botana, 2000; Lewis, 1986a; Lewis, 1986b). The local economy of the Pacific island nation of Kiribati was severely impacted after the loss of their export trade with Hong Kong due to an outbreak of ciguatera from their exported fish. This resulted in a loss of AU$250,000 annual income (Laurent et al., 2005). In Qld, Australia, Spanish mackerel and coral trout support commercially important fisheries and are known to accumulate ciguatoxins, however, the economic impact of ciguatera on these fisheries has not been explored. The Sydney Fish Markets (SFM), the largest fish market in the southern hemisphere, has imposed restrictions and bans on fish species and locations associated with high risk of ciguatera since a major outbreak of ciguatera in 1987. Restrictions prohibit the sale of certain fish at the SFM, particularly Spanish mackerel, supplied from Platypus Bay, Fraser Island, Qld. Since 2014 an increase in reported ciguatera cases in southern Qld has coincided with the migration of Spanish mackerel into these coastal waters. The first reports of ciguatera incidence in adjoining northern NSW was associated with the migration of Spanish mackerel from southern Qld tropical waters into

the temperate northern NSW waters (Farrell et al., 2016a; Farrell et al., 2016b). To truly reflect ciguatera-associated economic losses and health costs, a much greater, in-depth understanding of distribution and seasonality patterns, prevalence and incidence of ciguatera is required.

1.2.4 Occurrence of ciguatera

Annual reported ciguatera cases are often estimated from health and hospital records. Mild cases, however, are often not recognized and gastrointestinal symptoms experienced are frequently misdiagnosed as influenza or general food poisoning. Furthermore, affected travelers returning to temperate regions are often misdiagnosed as medical practitioners there are unfamiliar with this tropical illness. It has been estimated that fewer than 10 – 20% of ciguatera cases are actually reported to medical authorities in tropical regions (Arena et al., 2004; Lewis, 2006). Other factors that influence under-reporting include geographic isolation, inadequate access to medical and technical infrastructure (Anderson and Lobel, 1987; Kaly et al., 1991), and preferential use of traditional medicines (Kumar-Roiné et al., 2011). Reporting of ciguatera cases in Qld, Australia, is mandatory for medical staff and is collated and managed as part of the Australian government health database. These data are analysed in chapter 2 and compared with previous studies conducted between 1976 and 1995 (Gillespie et al., 1986; Harvey, 1997).

The health database was used in previous studies to investigate ciguatera distribution trends in Qld with the focus of the study being the origin (i.e. catch location) of fish implicated in reported ciguatera cases. Spanish mackerel

(Scombridae) and coral trout (Serranidae) have been frequently implicated in reported ciguatera cases since 1965, when relevant records were first kept (Gillespie et al., 1986). These earlier studies identified that serranids were more frequently implicated north of Rockhampton (23 °S), while scombrids were associated with ciguatera mostly south of this latitude (Gillespie et al., 1986; Harvey, 1997). Not all reports of ciguatera, however, included the fish species and/or origin of the fish, which may bias apparent distribution trends across Qld. Ciguatera occurrence based on the location of reported cases is likely to be documented more reliably on the health database. By contrast, identifying the origin and species of fish responsible for the ciguatera case is far more problematic. The health database has not previously been used to investigate potential spatial patterns of origin of fish responsible for ciguatera cases and regional location of reported cases in Qld. Diet and movement patterns of fish implicated in ciguatera in Qld often differ. Scombridae are predatory and migratory, serranids are predatory and sedentary reef fish (Begg and Hopper, 1997; Beukers-Stewart and Jones, 2004; St John et al., 2001). The potential vectors that transfer ciguatoxins into the diet of these mesopredators, and other fish species frequently implicated in ciguatera in Qld are unknown. Chapter 2, therefore, investigates regional distribution and spatial patterns of fish often implicated in ciguatera and as well as location of reported cases in Qld. Diets of fish families frequently implicated in ciguatera are also investigated and compared with the diets of fish families similarly implicated in the Pacific and Caribbean, to provide a broader understanding of potential vectors.

In the Pacific region, occurrence of ciguatera and *Gambierdiscus* populations have been positively linked to warmer sea surface temperatures (SSTs) during warm climate periods, such as El Niño and the Pacific Decadal Oscillation (PDO) (Hales et al., 1999; Llewellyn, 2010). The intensity of El Niño periods is thought to be influenced by the ocean-scale PDO, which oscillates between warmer than average (warm phase) to cooler than average (cool phase) cycles every 25 – 30 years (Chavez et al., 2003; Rongo and van Woesik, 2011). Warmer SSTs can also trigger coral bleaching, which provides new substrates for macroalgae and associated benthic dinoflagellates to colonise. Larger size of *Gambierdiscus* populations have been reported 13 – 17 months after warmer SSTs were recorded (Chateau-Degat et al., 2005), and within two to three months following coral bleaching events (Bagnis and Rougerie, 1992; Turquet et al., 2001). Environmental disturbances, including cyclones, storms and *Acanthaster planci* outbreaks also provide new substrates (dead coral surfaces) for increased abundance of macroalgae colonised by *Gambierdiscus* populations. Climate change conditions are predicted to increase the frequency of warmer climatic periods, with a greater risk of coral bleaching and occurrence and intensity of other environmental disturbances (Hoegh-Guldberg and Bruno, 2010; Hughes et al., 2017). It is likely that ciguatera incidence will increase under predicted climate change scenarios. Although reported ciguatera incidence was linked to warmer periods during the PDO, this relates primarily to the most recent PDO warm phase between 1976 and approximately 1998 (Llewellyn, 2010). The effect of the PDO, El Niño and environmental disturbances on ciguatera has not previously been investigated in Qld. Therefore a desk study was carried out (chapter 3) to evaluate whether

fluctuations in reported ciguatera cases were associated with such phenomena. Chateau-Degat et al. (2005) identified a timeline of approximately 13 – 17 months between warmer SSTs and increases in abundance of Gambierdiscus, with a further three months before increased incidence of ciguatera. It is therefore possible that ciguatera occurrence is lower in the year of coral bleaching. Whether delays are associated with preferences of *Gambierdiscus* for particular macroalgal substrates is unknown on the GBR.

1.2.5 Ciguatera-causing dinoflagellates

The principal dinoflagellate taxon responsible for ciguatera, the genus *Gambierdiscus*, occurs primarily on macroalgal substrates within benthic dinoflagellate assemblages that are often dominated by *Prorocentrum* and/or *Ostreopsis* species. Grazing fish species have been traditionally implicated as vectors in the uptake of ciguatoxins (Cruz-Rivera and Villareal, 2006; Hales et al., 1999; Heil et al., 2004), however, a recent feeding trial demonstrated the uptake of *Gambierdiscus* and bioaccumulation of ciguatoxins in the filter-feeding bivalves, *Tridacna maxima* (Roué et al., 2016). The potentially important role of benthic invertebrates in the trophic transfer of ciguatoxins has been raised previously (Cruz-Rivera and Villareal, 2006; Heimann et al., 2011; Lewis, 2006), but has received little attention. It is suggested that larger invertebrates, such as sea urchins and some crustaceans may inadvertently graze on benthic dinoflagellates. Such inadvertent ingestion has similarly been hypothesised for grazing fish. Other invertebrates, such as certain types of benthic zooplankton are likely to graze directly on benthic dinoflagellates (Figure 1.3). It is unknown whether substrate preference is related to macroalgal palatability of dominant grazers, such as remnant, unpalatable macroalgae remaining after grazing by

fish species, or whether substrate choice is to avoid direct benthic zooplankton predation.

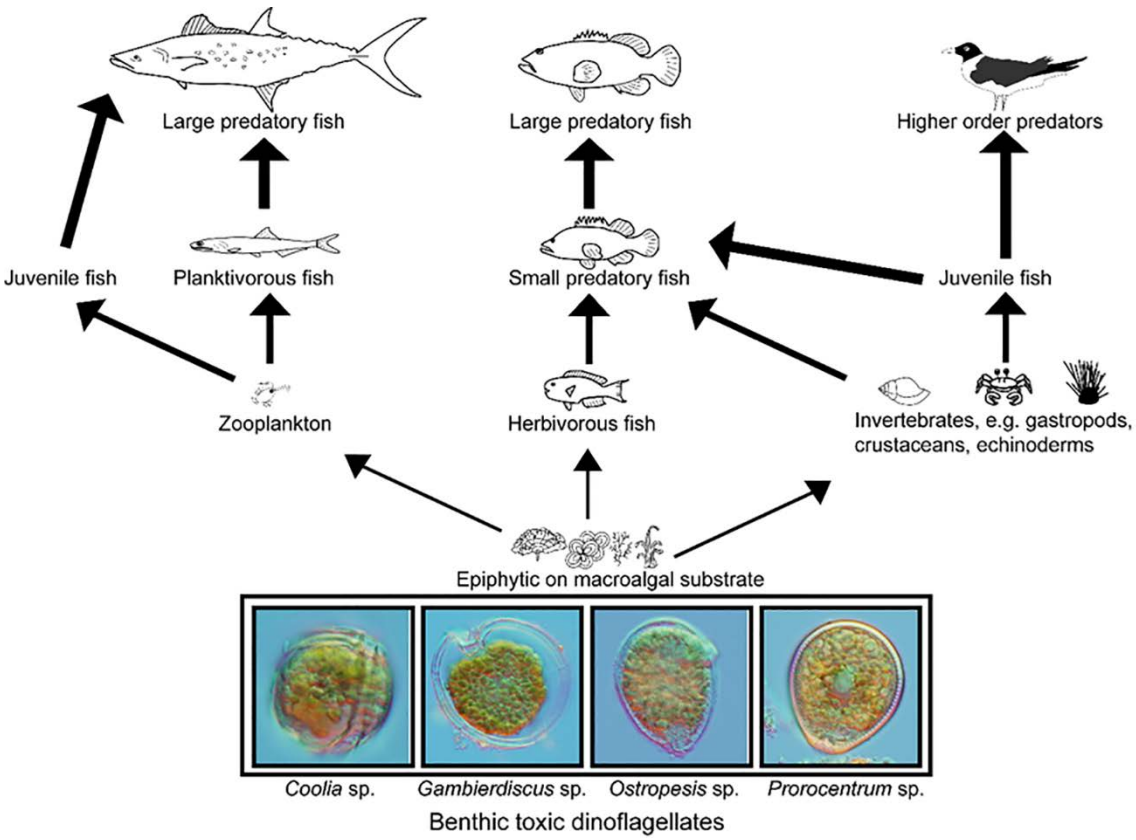

Figure 1.3 Schematic diagram of potential grazing vectors and potential trophic transfer routes of ciguatoxins (Heimann et al., 2011).

The sporadic and patchy distribution of *Gambierdiscus* populations on macroalgal substrates is thought to be governed by a complex interplay between factors affecting dinoflagellate growth (environmental and physico-chemical parameters) and predation/ grazing pressure. *Gambierdiscus* is not an obligate epiphyte, but has varied degrees of motility depending on availability and identity of macroalgal substrata (Parsons et al., 2011; Rains and Parsons, 2015). Motility may provide an advantage during periods of environmental stress (e.g. changes in salinity, temperature and light intensity), allowing *Gambierdiscus* cells to move between substrata and depth to minimise stress.

The size and distribution of *Gambierdiscus* populations on macroalgae vary among and within reefs, and among macroalgal species within a reef (Cruz-Rivera and Villareal, 2006). At present, ciguatera research focuses on sampling substrata with high abundances of *Gambierdiscus*. Such high abundances may reflect low grazing pressure on the dinoflagellates due to low herbivore abundance and thus potentially low rates of ciguatoxin transfer through marine food webs. Macroalgal palatability varies widely, with grazing preferences for algae by some fish and sea urchin species documented on the GBR (Mantyka and Bellwood, 2007; Rasher et al., 2013; Seymour et al., 2013). Potential substrate preferences of benthic dinoflagellates in relation to thallus form or grazing pressure on the GBR are unknown. As abundances of benthic dinoflagellates, including *Gambierdiscus* have not been recorded in the central GBR, field surveys were conducted (chapter 4) to investigate benthic dinoflagellates abundances and potential macroalgal substrate preferences of these dinoflagellates at inshore and mid-shelf reefs. There appeared to be within-reef specific preferences of dinoflagellates for macroalgal substrata, suggesting that environmental factors affect dinoflagellate abundance more strongly than type and availability of macroalgal substrates. In the absence of a macroalgal substrate preference, benthic dinoflagellates have the potential to expand into cooler coastal habitats, where macroalgae often dominate reef benthos.

Tropical coral reefs are dynamic ecosystems and temporary or permanent shifts in dominance from coral to macroalgal substrata have been attributed to environmental disturbances such as cyclones, *A. planci* outbreaks and coral

bleaching events triggered by SSTs (Cheal et al., 2010; Diaz-Pulido and McCook, 2002a). On the GBR freshwater influxes often follow cyclones and storms, resulting in reduced salinities sometimes < 10 in coastal habitats, such as estuaries and river mouths (Devlin et al., 2001). Freshwater plumes can cause osmotically-induced coral bleaching on inshore reefs extending sometimes as far off the coast as mid-shelf reefs (Devlin et al., 2001). Although environmental disturbances can lead to more frequent occurrence of ciguatera poisoning (chapter 3), the effect of temperature and salinity stressors on the growth of benthic dinoflagellate populations on the GBR is unknown.

Warmer SSTs experienced through climate change potentially create range expansions of dinoflagellate populations, not only within tropical regions but also from tropical to sub-tropical and even colder marine habitats (Heimann et al., 2011; Kohli et al., 2014). The expansion of *Gambierdiscus* populations into temperate regions has been recorded since 2003 (Figure 1.2). Recently, *Gambierdiscus carpenteri* was recorded in southern NSW waters off Merimbula (Kohli et al., 2014; Rhodes et al., 2014). The East Australian Current (EAC), which has been strengthened under climate change conditions, (Heimann et al., 2011) is the likely vehicle for this range expansion. It has been demonstrated that the EAC provides seasonal replenishment of reef fish populations from southern Qld to more southerly estuarine environments during the Austral summer (Booth et al., 2007). There is evidence that tropical reef fish are acclimating to cooler temperatures in these southern habitats and thereby surviving as permanent populations (Figueira and Booth, 2010). However, it is unknown whether *G. carpenteri* populations are also persisting in NSW

coastline. It is also unknown whether species occurring in temperate regions produce toxins and which factors regulate toxin production. It is therefore important to identify and understand responses of ciguatera-causing dinoflagellates to environmental drivers as these drivers are likely to govern any range expansion into temperate regions. Along with *G. carpenteri*, other benthic dinoflagellates of unknown origin were documented in southern NSW waters (Kohli et al., 2014). There has been little focus, however, on the influence of benthic dinoflagellate assemblages on *Gambierdiscus* populations and their potential importance for survival under changing climatic conditions. In chapter 5, the effects of temperature and lower salinities were investigated on population growth of two strains of *G. carpenteri* isolated from GBR waters. One strain was from an inshore habitat and the other from a more stable marine environment, similar to offshore habitats. Differences in population growth responses to temperature and salinity changes between the two strains suggest that habitat of origin may influence ability to persist in changing environmental conditions (Sparrow et al., 2017). As *Gambierdiscus* co-occurs predominantly with *Prorocentrum* and *Ostreopsis* species on substrates in the central GBR (investigated in chapter 4), the effect of mixed benthic dinoflagellate assemblages on growth rates of *G. carpenteri* at lower salinities was also investigated. Salinity had a significant effect on the survival of *G. carpenteri*, which increased with lower salinities for the inshore isolate. These data suggest a likely potential for southward range expansion of benthic dinoflagellates into colder coastal environments, including estuaries.

In Merimbula, southern NSW, *G. carpenteri* populations occur from January to May, however, the presence of the species did not result in visible benthic dinoflagellate blooms during this period (Kohli et al., 2014). This observation suggests that environmental conditions in southern NSW either limit growth (prevent blooms) or that populations are actively grazed by resident fish species. While ciguatoxins bioaccumulate in marine food webs, it is unknown whether macroalgal substrates with large population sizes of benthic dinoflagellates are targeted by grazing fish compared to less populated macroalgal substrates. Planktonic dinoflagellates and diatoms are a primary source of essential long-chain polyunsaturated fatty acids (LC-PUFAs) in marine food webs (Carreón-Palau et al., 2013; Kelly and Scheibling, 2012; Litz et al., 2010), however, there is a paucity of research on the nutritional value of benthic dinoflagellates (Usup et al., 2008). Particularly important for healthy human diets are the essential fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Fernandes et al., 2014; Huynh and Kitts, 2009; Sargent et al., 1999). As these fatty acids cannot be biosynthesized by vertebrates, they must be taken up in the diet (Carreón-Palau et al., 2013; Li et al., 2012). The nutritional qualities of benthic dinoflagellates could be affected by temperature and salinity changes. As small-scale experiments determined that temperature and salinity affected growth rates of *G. carpenteri* populations (chapter 5), large-scale cultures of *G. carpenteri*, *Prorocentrum lima* and *Ostreopsis* sp. were raised to investigate whether lower temperature and salinity would affect biochemical profiles (chapter 6). To simulate bloom conditions, cultures were supplemented with nitrate and/or phosphate when needed and their growth rates and nutrient uptake rates were monitored.

Population growth of *G. carpenteri* was reduced by lower salinity. Population growth of *Ostreopsis* sp. was reduced by lower temperature. In comparison, population growth of *P. lima* was not affected by temperature or salinity. This suggests that potential for southward range expansion into cooler coastal habitats is likely to differ among benthic dinoflagellates, with *P. lima* capable of adapting to a wider range of coastal habitats compared to *G. carpenteri* and *Ostreopsis*. While estuaries and riverine environments appear to be unsuitable for colonies of G*. carpenteri* and *Ostreopsis* to populate in the tropics, populations have been recorded in these environments in southern NSW (Heimann et al., 2011; Kohli et al., 2014). Whether these benthic dinoflagellates have been transported from the southern GBR to NSW by the EAC, as has occurred for some reef fish, and are adapting to local climatic and environmental conditions is still unclear and needs further research.

In summary, the present state of knowledge is reviewed in chapter 2, whilst research relating to the focus of the thesis is presented in chapters 3 – 6. Research outcomes are synthesised and future areas of research focus are identified in the general discussion (chapter 7).

This thesis focused on research gaps in the occurrence of ciguatera in Queensland, Australia relative to:

- climate change and oceanic ecosystem trends;
- investigation into macroalgal substrate preference of benthic toxic dinoflagellate assemblages in the central Great Barrier Reef;

- population growth responses of GBR-isolates of *Gambierdiscus carpenteri* to temperature and salinity, including the potential role of co-occurring benthic dinoflagellates to shape responses under adverse conditions associated with climate change; and
- impact of temperature and salinity on nutritional quality (essential LC-PUFA, critical for marine food webs), nutrient uptake rates and growth of benthic dinoflagellates.

Brief descriptions of the aims for each chapter are provided below.

The objective of chapter two was to evaluate the present state of knowledge on ciguatera in Qld, Australia. Therefore, the aims of chapter two were to:

- investigate regional distribution patterns of reported ciguatera cases;
- identify fish families frequently implicated in reported cases;
- investigate spatial trends in fish families implicated in reported ciguatera cases;
- analyse the reported diets of fish families frequently implicated in ciguatera outbreaks to identify potential key vectors in the trophic transfer of ciguatoxins; and
- compare a 20-year study (1976 – 1995) of ciguatera incidence with current accessible data for 1996 – 2012.

The objective of chapter three was to evaluate whether fluctuations in the occurrence of ciguatera in Qld, Australia are associated with climatic periods or environmental disturbances, through a unique study of reported cases covering a 37-year period. Therefore, the aims of chapter three were to:

- determine whether El Niño and/or the PDO affects the frequency of ciguatera incidence;
- evaluate the effect of environmental disturbances on ciguatera incidence;
- determine whether coral bleaching interacts with warmer climate periods in relation to incidence of ciguatera.

Chapter four focused on improving current knowledge of benthic toxic dinoflagellate population size and structure on macroalgal substrates in the central Great Barrier Reef. Therefore, the aims of chapter four were to:

- investigate potential seasonal or temporal trends in the occurrence of benthic toxic dinoflagellates;
- investigate grazing and macroalgal morphology as potential drivers in the distribution of benthic toxic dinoflagellates.

Chapter three determined that ciguatera incidence increases in the warm PDO phase, but incidence is low in the actual year of coral bleaching, suggesting environmental stressors, such as temperature and salinity may affect growth of the relevant dinoflagellates. The objective of chapter five was to investigate growth responses of two strains of *G. carpenteri* and its associated dinoflagellate assemblage to changes in temperature and salinity. This

knowledge is critical to determine potential range expansion under changed climatic conditions. Therefore, the aims of chapter five were to:

- investigate population growth of two strains of *G. carpenteri* in lower salinities at three temperatures, typical of environmental conditions in coastal waters and on coral reefs; and
- investigate effects of mixed benthic dinoflagellate assemblage structure on population growth and survival of *G. carpenteri* in lower salinities.

Ciguatera requires ingestion of *Gambierdiscus* spp, which may well be governed by “tastiness” of the prey. As small-scale experiments demonstrated potential for range expansion, chapter six investigates the effect of temperature and salinity on the nutritional value of benthic toxic dinoflagellates (particularly content of essential LC-PUFA). This knowledge is essential in predicting the likelihood of ciguatera range expansion to cooler southern Australian climates. Therefore, the aim of chapter six was to:

- determine the effect of temperature and salinity on biochemical profiles and growth potential of *G. carpenteri* and its associated dinoflagellate assemblage.

Chapter Two: Spatial patterns and occurrence of ciguatera in relation to commercially important fisheries in Queensland, Australia (1976 – 2012)[2]

2.1 Abstract

Ciguatera is an illness caused by ingestion of ciguatoxins in fish, which affects coastal communities in tropical regions. It is predicted that ciguatera incidence in tropical regions will increase under climate change conditions, while coastal populations in colder regions may be increasingly at risk of ciguatera due to extended migration patterns of fish with accumulated ciguatoxins under climate change-induced weather conditions. Ciguatera is frequently reported in Queensland (Qld) Australia, however, the full impact of ciguatera on coastal populations has not been explored. In this study, the Qld Health Department database for foodborne diseases was used to investigate geographical distribution, potential seasonality of ciguatera cases and to identify fish families frequently implicated in ciguatera cases. The diet of fish families most often associated with ciguatera for Qld, the Pacific and Caribbean regions was identified using a publicly accessible fish database. Reported cases and fish families implicated in ciguatera showed regional variation in Qld. Occurrence of ciguatera was lower in isolated, less populated communities in central and far north Qld, while the larger number of reported cases in south-east Qld between 1996 and 2010 appeared to drive temporal occurrence. Seasonal peak during

2 The paper was written in its entirety by Leanne Sparrow with editorial input by Kirsten Heimann, Leone Bielig, Angela Capper and David Blair.
The work is presented as submitted, except for changing the formatting to match that of the rest of this thesis.

the Austral wet season was driven by Scombridae, with 75% of cases reported for this fish family observed in this season. By contrast, Serranidae were only implicated in cases reported north of 25 °S, which may be habitat-associated. Fish prey and benthic crustaceans were major dietary components of mesopredators, such as scombrids and serranids, which were often associated with ciguatera throughout the tropical regions, including Qld. Lower number of reported cases with distance from the highly populated south-east may be skewed by under-reporting, which may also effect distribution patterns of fish families frequently implicated by ciguatera. It is therefore important to implement a questionnaire that can obtain qualitative and quantitative data to provide a more accurate understanding of the impact of ciguatera, while ecological research would contribute knowledge to potentially significant prey in complex food webs that leads to accumulation of high ciguatoxin concentrations in mesopredators.

2.2 Introduction

Humans can be affected by ciguatera poisoning when they consume fish that have accumulated ciguatoxins through their diet. Ciguatoxins are derived primarily from the benthic dinoflagellate, *Gambierdiscus*, which often co-occurs with other benthic dinoflagellates mostly on macroalgal substrates, but also on coral and sand (Faust, 1995; Litaker et al., 2009; Morton and Faust, 1997). Warmer sea surface temperatures (SSTs) have been linked to increases in abundance of *Gambierdiscus* populations and consequent increases in ciguatera poisoning in humans (Chateau-Degat et al., 2005; Llewellyn, 2010). Furthermore, *Gambierdiscus* species are less tolerant of lower temperatures at

higher latitudes (Kibler et al., 2012; Xu et al., 2014), similar to the thermal tolerance of corals with latitude recorded for corals on the Great Barrier Reef (GBR) (Berkelmans et al., 2012), and foraminifera along the Queensland (Qld) coastline, Australia (Momigliano and Uthicke, 2013). As the GBR and Qld coastlines extend more than 12 and 18° of latitude, respectively, it is predicted that *Gambierdiscus* populations will be less tolerant of cooler temperatures at higher latitudes and thus, ciguatera occurrence could decline at higher latitudes along the Qld coastline.

Misdiagnosis of ciguatera illness is the primary factor for under-reporting, exacerbated by geographic isolation and inadequate access to medical infrastructure in remote tropical regions (Anderson and Lobel, 1987; Kaly et al., 1991). This can lead to vast differences in estimates of numbers of cases, as observed among island nations in the Pacific region (Lewis, 1986b; Skinner et al., 2011). In Qld, only temporal patterns of ciguatera poisoning, based on annual reported cases, have been documented, although it was suggested that under-reporting appeared to increase with distance from metropolitan south-east Qld (Gillespie et al., 1986). Regional distribution of ciguatera poisoning has not previously been investigated in Qld. Instead, distribution patterns of ciguatera for Qld focussed on the origin of fish implicated in ciguatera cases, with emphasis on scombrids and serranids (Gillespie et al., 1986; Harvey, 1997).

It was first hypothesised by Randall (1958) that grazing fish inadvertently uptake *Gambierdiscus* while grazing on macroalgae. Recent research supports this general hypothesis with the discovery of ciguatoxins in grazing Acanthuridae (surgeonfish) and Labridae (parrotfish) (Chinain et al., 2010). Dinoflagellates, however, are a rich source of essential fatty acids (Carreón-Palau et al., 2013; Kelly and Scheibling, 2012; Wilson et al., 2001), it is unclear whether macroalgae colonised by benthic dinoflagellates are directly targeted by grazing fish. Although grazing fish species are a variable component of the diet in many Pacific Island nations, mesopredators are a common group of fish implicated in ciguatera (Baumann et al., 2010; Morris et al., 1982; Rongo and van Woesik, 2011; Stinn et al., 2000). By contrast, only mesopredators have caused ciguatera in Qld. The main reason for this is that few people in Qld target and eat grazing reef fish in Qld. The main targets of reef fishing in tropical Qld are Serranidae (coral trout and grouper) and Scombridae (mackerel), both frequently implicated in reported ciguatera cases (Gillespie et al., 1986; Harvey, 1997). Although it is assumed that ciguatoxins bioaccumulate through marine food webs by trophic transfer, from grazing fish, such as surgeon- and parrotfish, to mesopredators, this link has not been confirmed.

In Qld, recreational, commercial and charter fishing combined have been estimated at an annual gross value between AU$43 and 60 million (Innes et al., 2014a; Jones et al., 2007; Thébaud et al., 2014). Most commercially targeted fish in Qld are sourced from the GBR and are known sources of ciguatera. Although serranids and scombrids continue to be frequently associated with ciguatera in Qld, these species remain important fisheries to the Qld economy.

(Innes et al., 2014a; Thébaud et al., 2014). Management practices in Qld are currently dependent on bans, restrictions and conservation legislation to reduce the risk of ciguatera occurrence. Particular species of tropical snappers (Lutjanidae) are known carriers of ciguatoxins and are considered high-risk of causing ciguatera poisoning in humans in Qld. For this reason, the Qld Department of Agriculture and Fisheries (DAF) imposed a ban on the keeping or sale of red bass (*Lutjanus bohar*), paddletail (*L. gibbus*) and chinamanfish (*Symphorus nematophorus*) (Gillespie et al., 1988). Fisheries restrictions that may also reduce the risk of ciguatera poisoning, including maximum length limits for certain species. Furthermore, bans on sale of certain fish species (e.g. Spanish mackerel) from particular locations in Qld and from certain locations in the Pacific have been implemented by the Sydney Fish Market (SFM), New South Wales (34°S), the largest fish market in the southern hemisphere. These restrictions on sale of certain species from certain locations were in response to an outbreak of ciguatera that affected 63 people in Sydney due to consumption of Spanish mackerel sourced from Qld coastal waters in Platypus Bay, Fraser Island (25 °S) (Capra and Cameron, 1990). A Humphead Maori wrasse (Labridae), which was sourced from GBR waters, was the cause of a ciguatera outbreak in Victoria (37 °S) in 1997 (Ng and Gregory, 2000), and Maori Wrasse have also been implicated in three ciguatera incidents in Qld prior to 1984 (Gillespie et al., 1986). This is a sought-after fish species in the Pacific region, but is now protected from fishing in Qld waters as an iconic species on the GBR under Australian and Qld state conservation legislation.

Spatial management zones, fish bans and weight limits can affect the rate and distribution patterns of ciguatera incidents. It is therefore critical to ensure best management practices can be applied to minimise the socio-economic risk of ciguatera to Qld fisheries. Literature to date on the status of ciguatera in Qld, however, lacks the spatial resolution to identify Qld-wide patterns of ciguatera distribution, particularly in terms of ciguatera cases and, to a lesser extent, spatial sources of implicated fish (e.g. Gillespie 1988, Harvey 1997). It is important to define the spatial distribution of cases and sources of ciguatera, since the potential for increases in SSTs in the future due to climate change raise the prospect of such distributions changing, specifically becoming more prevalent at higher latitudes. The aims of this study were to review the current state of knowledge on ciguatera occurrence in Qld between 1996 and 2012, and investigate seasonal and regional patterns of distribution of ciguatera based on case locations as well as the origin of fish implicated in these cases. Current knowledge was compared with available data on annual reported cases and origin of implicated fish from an earlier 20-year study (1976 – 1995) (Harvey, 1997); data by Gillespie 1965 – 1984 (Gillespie et al., 1986) could not be included because data records prior to 1976 were not reliable and annual reported cases of ciguatera were not detailed prior to 1976.

2.3 Methods

In Australia, hospital and health records are collated into a database maintained by the Australian Government Department of Health. As reporting of ciguatera cases is only mandatory in Qld, occurrence and distribution of ciguatera has not been investigated in any other Australian state. Due to the length of the Qld

coastline, distribution of ciguatera cases was subdivided between northern and southern Qld areas and further sub-divided into five zones as defined by Harvey (1997) (Figure 2.1). Distribution patterns of ciguatera cases in previous studies had access to the origin of fish implicated in the particular ciguatera case in

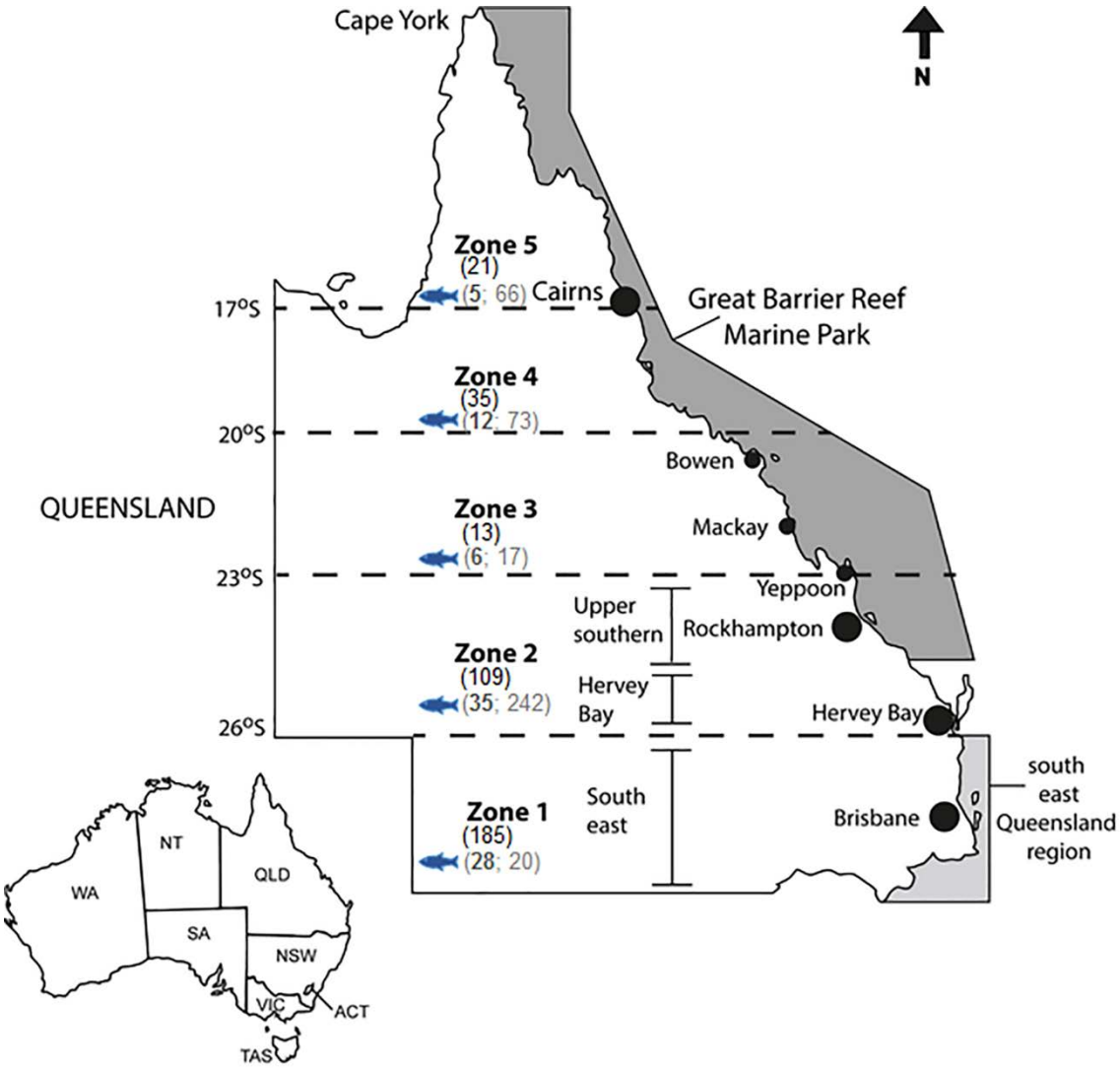

Figure 2.1 Map of Queensland (Qld), Australia. Yeppoon lies at the boundary between northern and southern Qld (23°S); the metropolitan south-east region is shown. Five regional zones (as derived by Harvey 1997) are indicated: zone (1) south of 26°S; (2) north of 26°S to 23°S (north of Yeppoon); (3) north of 23°S to 20°S (south of Bowen); (4) north of 20°S to 17°S (south of Cairns); (5) north of 17°S. Number of cases reported in each zone during the study period between 1996 to 2010 shown in brackets below zone number, and figures shown in brackets on right of fish symbol in each zone indicates number of reported cases based on locality of fish capture in the period 2001 – 2012 and in the period 1976 – 1995 (Harvey 1997), respectively.

45% of cases (Table 2.1). In the present study, origin of fish implicated in any particular ciguatera case was recorded for only 22% of cases reported between 1996 and 2012, which indicates that greater care in recording connected data was taken in earlier recordings of ciguatera incidence. By contrast, location of the ciguatera case itself was available for 94% of cases reported (Table 2.1) and was, therefore, used to investigate regional distribution patterns of cases.

Table 2.1 A comparison of studies on ciguatera occurrence in Qld where reported cases included the regional case location and where implicated fish were caught.

No. cases reported	Current study 1996 – 2012	Gillespie et al (1988) 1965 – 1984	Hervey (1997) 1976 – 1995
Total – Qld	385	527	925
Case location included (%)	363 (94)	-	-
Fish location included (%)	86 (22)	258 (49)	418 (45)
Southern Qld (%)	67 (78)	218 (84)	262 (63)
Northern Qld (%)	19 (22)	40 (16)	156 (37)
Location – no data (%)	299 (78)	269 (51)	507 (55)

This study utilised publicly available databases. The Ozfoodnet Working Group database produced quarterly information on the reported cases of foodborne diseases in Australia, including ciguatera, for the period, 2001 - 2012. These data were supplemented with information from Queensland Health for the period, 1996 – 2000, however, information on fish implicated in reported ciguatera cases for Qld, within the study period, could only be obtained from

2001 onwards. When available, records detailed the identities of the fish species consumed, location (origin) of fish, and source, i.e. whether fish were caught recreationally or purchased in restaurants, fish shops or supermarkets.

Fishbase (Froese and Pauly, 2010) incorporates records of fish associated with ciguatera incidents or analysed for ciguatoxins. For the South Pacific, the database incorporated relevant information provided by the Secretariat of the Pacific Community (SPC, previously known as the South Pacific Commission), for the year 1990. The origin, geographic distribution and frequency of occurrences of ciguatera cases were detailed, when known. All fish species implicated in ciguatera cases according to both databases were identified to family level along with their common names (Table 2.2). Dietary analysis of fish families associated with ciguatera in Qld were limited in Fishbase (Froese and Pauly, 2010), so studies from the Pacific and Caribbean regions were incorporated for a broader understanding of potentially important marine taxa in the trophic transfer of ciguatoxins to mesopredators.

Table 2.2 List of fish families implicated with reported human ciguatera cases in Queensland (Qld), Australia. The common names, as used in Qld, Australia are listed.

Fish Family	Common Name
Carangidae	Trevally, jack, pompano, scad, amberjack
Carcharhinidae	Requiem shark
Haemulidae	Grunts, sweetlips
Labridae	Wrasse
Lethrinidae	Emperor bream, emperor, sea bream
Lutjanidae	Snapper, red snapper
Mugilidae	Mullet
Pomatomidae	Bluefish, tailor
Sciaenidae	Jewfish
Scombridae	Mackerel, tuna, bonito
Serranidae	Coral trout, grouper, cod, sea bass
Sphyraenidae	Barracuda
Terapontidae	Grunter, tigerperch

Uranoscopidae	Stargazer

2.4 Results

2.4.1 Distribution of reported ciguatera cases in Queensland

Although a general decline in the occurrence of reported ciguatera cases was observed with northward distance from metropolitan south-east Qld, it was noted that the lowest frequency was recorded in Zone 3, in central Qld (Figure 2.1). Basing ciguatera occurrence on locality of fish capture, however, showed no latitudinal trend for the current or previous (1976 – 1995) study period. An infrequent occurrence of reported ciguatera by incidence and by locality of fish capture in Zone 3 was consistent over the 37-year period (Figure 2.1). Annually reported data available between 1996 and 2010, and based on case location, showed a general declined northward from Zone 1, apart from the lowest frequency of ciguatera in central Qld (Figure 2.2).

Despite massive inter-annual variability of case numbers, reported cases of ciguatera poisoning in humans per year declined markedly over the 37-year period of 1976-2012 in Qld (Fig. 2.2), from a high of almost 100 cases in 1979 to a low of 4 in 2012 (Fig. 2.2). Some regional differences in ciguatera frequency between 1996 and 2010 were observed, with most cases occurring in Zones 1 and 2 (Figure 2.2). Reported cases in the south-east region (Zone 1) dominated cases numerically for the whole of Qld. Within the remaining zones, reported cases were more frequent in zones two and four prior to 2005, but more frequent in Zone 5 after 2005 (Figure 2.2). The reported ciguatera cases

in south-east Qld (Zone 1) drives temporal trends for all of Qld combined, likely due to the much larger human population in Zone 1.

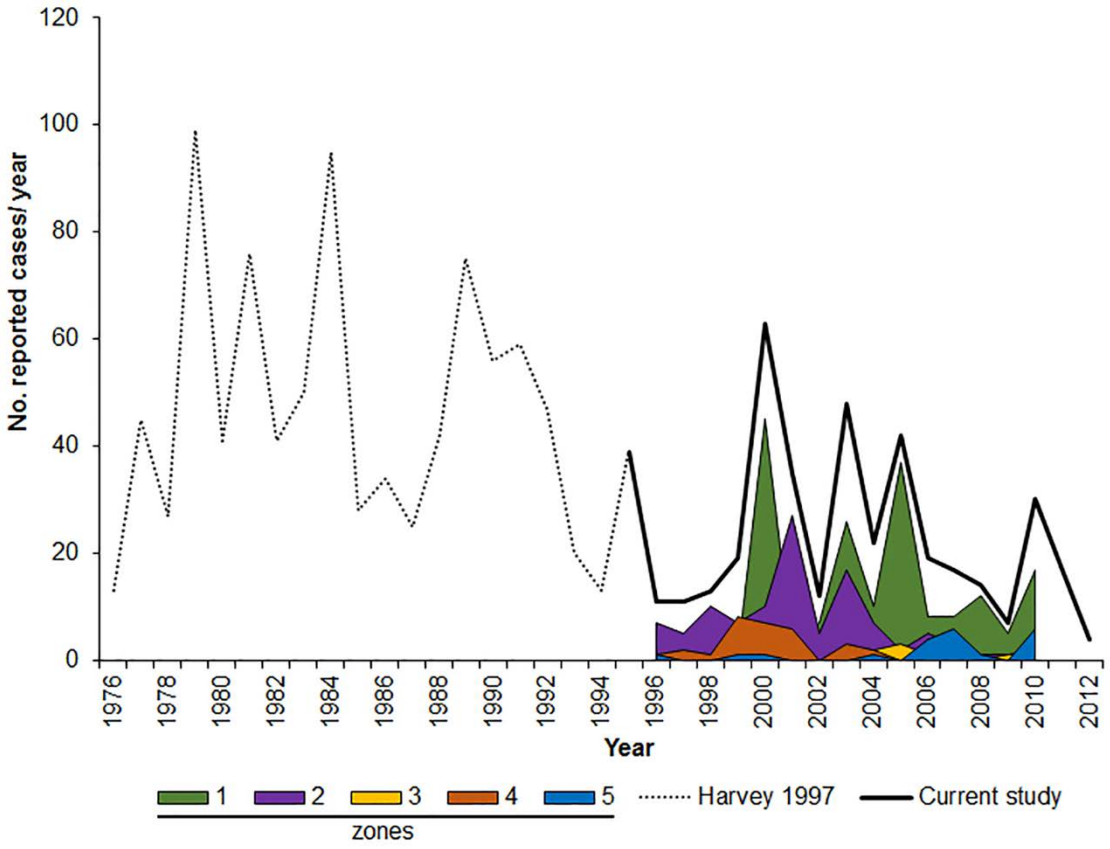

Figure 2.2 The frequency of reported cases of ciguatera poisoning in humans in Queensland (Qld), Australia over a 37-year period, 1976 – 2012 (adapted from Harvey 1997). Reported cases/ year are shown for the 20-year case study (Harvey 1997) and the 17-year period of the current study.

2.4.2 Diversity of fish families implicated in reported ciguatera, Qld

From 2001 until 2012, 90% of reported ciguatera cases listed the fish species associated with the case. To investigate trends in these data, they have been grouped into fish families. Number of fish families implicated in reported ciguatera cases was slightly higher (n = 10) in the previous study from 1976 to 1995 (Harvey, 1997) than in the current study (n = 8) (Figure 2.3). Scombridae and Serranidae have remained the most frequently implicated fish families in ciguatera poisoning of humans in Qld. By contrast, Carangidae appear to have

increased in frequency from approximately 3% to 16% between the studies (Figure 2.3). For this reason, the implicated fish families investigated further are Scombridae, Serranidae and Carangidae, with remaining fish families grouped together due to low occurrences.

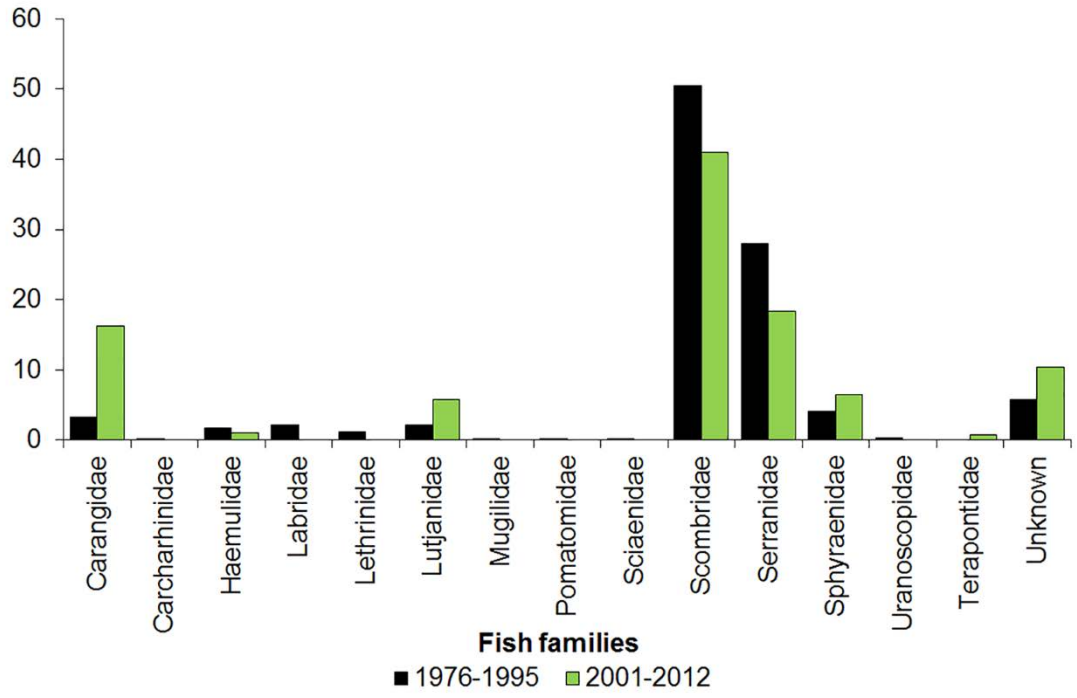

Figure 2.3 Percent of fish families implicated with reported ciguatera cases in Queensland for the period 1976 – 1995 (Harvey 1997) and for the period 2001-2012 (current study).

2.4.3 Seasonality of fish families implicated in reported ciguatera, Qld

Data for seasonal trends on which fish families were implicated in ciguatera cases were available only for the current study (2001-2012). The frequency of reported cases was higher in months associated with the Austral wet season, with 65% of cases reported in the wet season between November and April (Figure 2.4A and B). It is suggested that seasonality of cases was driven by capture of Scombridae, with 75% of cases that implicated this fish family being reported in the Austral wet season (Figure 2.4C). On a monthly basis,

Scombridae was frequently associated in the months of the wet season except for December (Figure 2.4A). The other fish families showed less seasonality in

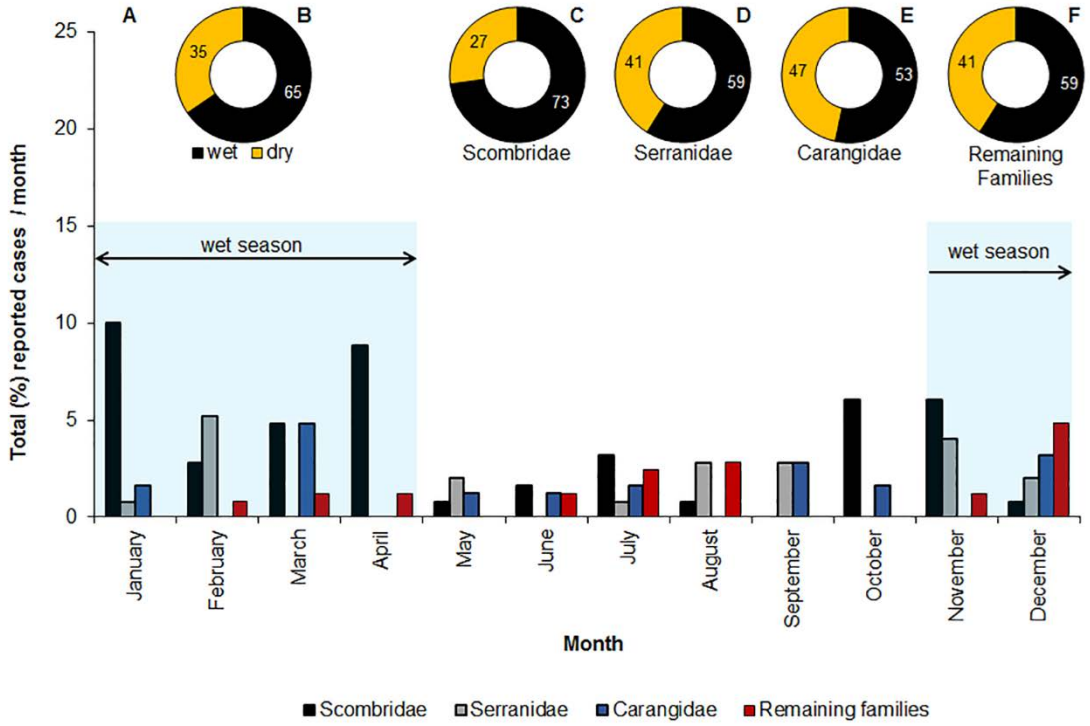

Figure 2.4 Percent monthly distribution of reported ciguatera cases in Queensland (Qld) for implicated fish families, Scombridae, Serranidae, Carangidae and remaining families during the current study, 2001 – 2012 (A). The percent seasonal distribution for reported ciguatera cases in Qld based on the Austral tropical wet season (November - April) and dry season (May – October) for the current study 1996-2012 (B); and associated with fish families (2001 – 2012): Scombridae (C); Serranidae (D); Carangidae (E); and remaining families (F).

their contribution to cases between the tropical wet and dry seasons (Figure 2.4C – E), although there was a slightly greater frequency during the wet season. Scombridae were implicated in reported cases for all months of the year, except September, while other fish families were not identified in four of the months during the study period (Figure 2.4A). The month with the highest frequency of cases occurred within the wet season for all fish families. Serranidae was most frequently associated with reported cases in February (5%) and November (4%) when Carangidae were not. In March, Carangidae

were most often reported with ciguatera cases while remaining families were most frequent in cases in December when Scombrids were infrequent (Figure 2.4A).

2.4.4 Regional trends of fish families implicated in reported ciguatera, Qld

The regional importance of fish families implicated in ciguatera cases in Qld was evaluated by the five zones (Fig. 2.1), as well as by northern and southern Qld areas, latitude 23 °S being the boundary between them (Figure 2.1). Scombridae were most commonly implicated in reported cases of ciguatera in southern Qld (52% of cases), although no regional (zonal) distribution trend was observed for this family (Figure 2.5A and B). Frequency of Serranidae implicated in reported ciguatera cases increased regionally, from south to north (Fig. 2.5A). In the south, Serranidae was not implicated in Zone 1, yet constituted 60% of implicated fish taxa in the far north, Zone 5 (Figure 2.5A), and 52% of fish implicated in the combined northern Qld zones (Figure 2.5C). Carangidae was also implicated at increasing rates from south to north increase from Zones 1 to 3, but was then absent from Zones 4 and 5 in ciguatera (Figure 2.5A). Carangidae was the most frequently implicated fish family in reported ciguatera cases in Zone 3, and this was the only zone in which Scombridae was not implicated in ciguatera. Remaining families were recorded only in Zones 1 and 4, but were the dominant (47% of cases) family implicated in the south-east region (Zone 1) (Figure 2.5A).

Locality of fish capture was not identified in reported cases prior to 2005, except for one incident involving three people in 2003. Between 2005 and 2012, locality

of fish capture was provided for 52% of reported cases (Supplementary Table S2.2, Appendix B). Although almost 50% of reported ciguatera cases were associated with a known source of fish (purchased or recreationally caught) only 31% included details of the locality of fish capture. Ciguatera cases associated with purchased fish declined from Zone 1 in the south (45%) to Zone 5 in the north (5%) (Figure 2.6A, B). Ciguatera cases associated with recreationally caught fish also showed a declining trend from southern to northern zones, being high in Zone 2 (~57%) and low in Zone 5 (~10%), but absent from Zones 1 and 3 (Figure 2.6A). The numbers of ciguatera cases were heavily biased (81%) toward purchased fish in the south (Fig. 2.6B) but were more evenly balanced in the north among purchased fish (44%) and recreationally caught fish (56%) (Figure 2.6C).

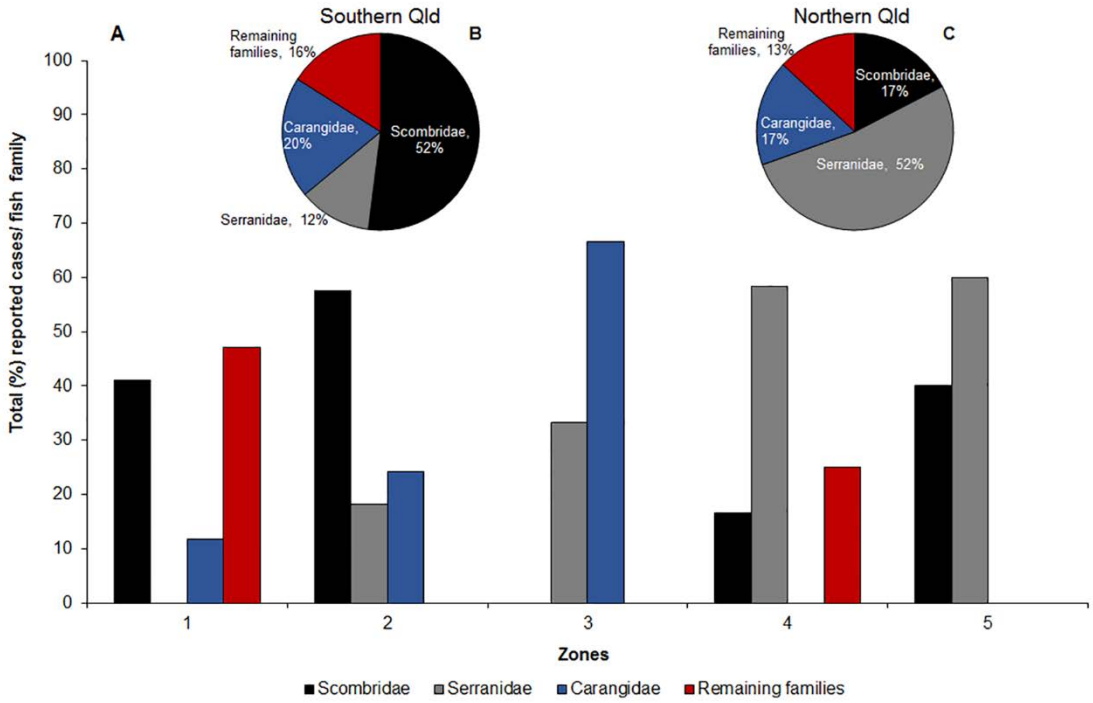

Figure 2.5 Percent distribution of reported ciguatera cases in the five Queensland (Qld) zones (see Figure 1) for the period 2001 – 2012 associated with: fish families, Scombridae, Serranidae and remaining families (A inserts: associated with fish families in southern Qld (Zones 1 and 2) (B) and in northern Qld (C).

As the southern area appeared to have a major influence on ciguatera occurrence for the whole of Qld (Figure 2.2) and differences between zones within the southern Qld area were evident (Figures 2.2, 2.5A and 2.6A), the southern area (Zones 1 and 2) was further divided into 3 sub-zones (South-East (Zone 1), Hervey Bay (Zone 2a), Upper Southern (Zone 2b); Fig. 2.7). The proportion of ciguatera cases declined from southern to northern sub-zones (Figure 2.7A). In the south-east sub-zone, only fish from purchased sources were implicated in ciguatera cases, with remaining families and Scombridae most often identified (Figure 2.7A and B). By contrast, recreationally caught fish were the primary sources of reported ciguatera cases in Hervey Bay, and the main vector was Scombridae, at 70% (Figure 2.7A and C). Serranidae was the only fish family associated with ciguatera cases in the Upper Southern Zone 2b (Figure 2.7D). The southern end of the GBR Marine Park is within this Upper Southern Zone 2b (Figure 2.1), and has suitable coral reef habitat for serranids. This highlighted the important transition of dominant vectors of ciguatera between the Hervey Bay and Upper Southern sub-zones.

2.4.5 Dietary composition of fish families of high ciguateric risk

Composition of the diet of fish associated with ciguatera cases was investigated to determine whether likely vectors in the transfer of ciguatoxins through marine food webs to mesopredators could be identified. In Fishbase (Froese and Pauly 2010), limited data was available on the diet of fish reported with ciguatera in Qld. Thus, dietary information for such fish from the Pacific and Caribbean regions were examined. This allowed identification of the six most frequent fish families associated with ciguatera cases throughout the tropical region: Serranidae, Scombridae, Lutjanidae, Sphyraenidae,

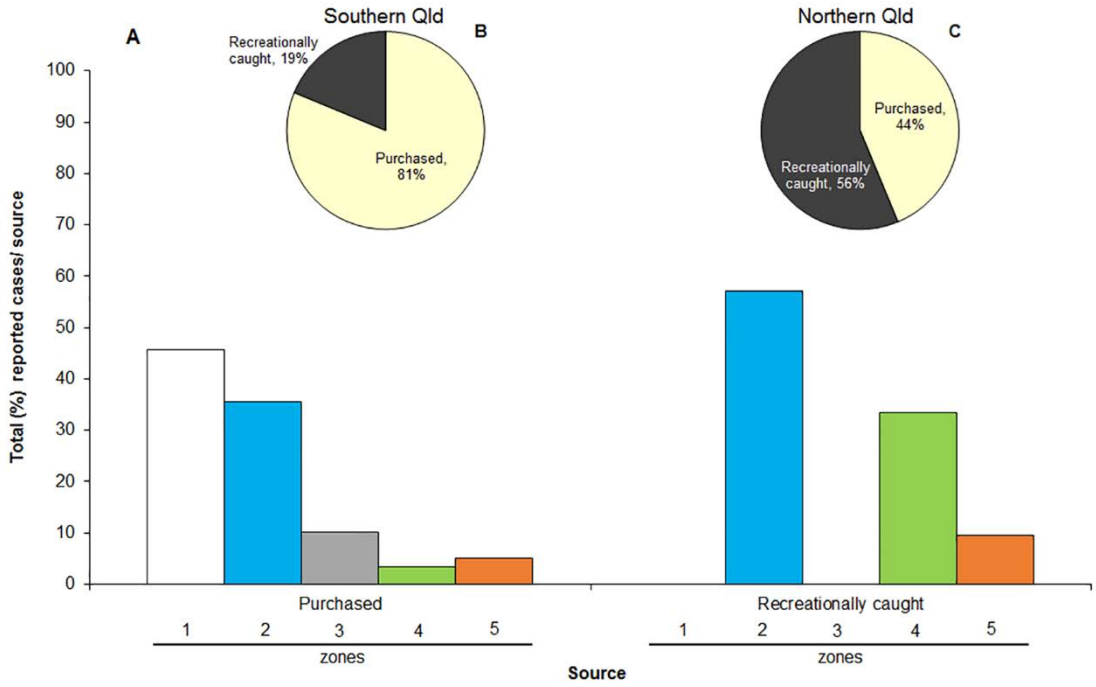

Figure 2.6 Percent of reported ciguatera cases in the five Queensland (Qld) zones (see Figure 2.1) for the period 2001 – 2012 associated with source of fish as purchased (restaurant, fish market or supermarket) or recreationally caught (and consumed) (A); Source of fish in southern Qld (zones 1 and 2) (B); and in northern Qld (zones 3 – 5) (C).

Carangidae and Acanthuridae (Supplementary Figure S2.1, Appendix C). Benthic crustaceans and fish were identified as major components of the diets of these families – with the exception of Acanthuridae where algae were the main dietary component identified (by Fishbase) (Figure 2.8A – C). The dominance of fish prey in the diet of Scombridae in Qld was similar to the diet of Scombridae in the Caribbean (Figure 2.8A and C). Fishbase (Froese and Pauly, 2010) contained only one study of diet of Serranidae in Qld (Figure 2.8A), which showed that fish and benthic crustaceans are major prey, which differed to results for this family in the Pacific and Caribbean, where other invertebrates, particularly molluscs, were an important minor component of serranid diets (Figure 8B and C). Carangidae and Lutjanidae were also frequently implicated

in ciguatera cases in Qld (Figure 2.3), and their diets consisted of a range of fish and invertebrates (Figure 2.8A – C).

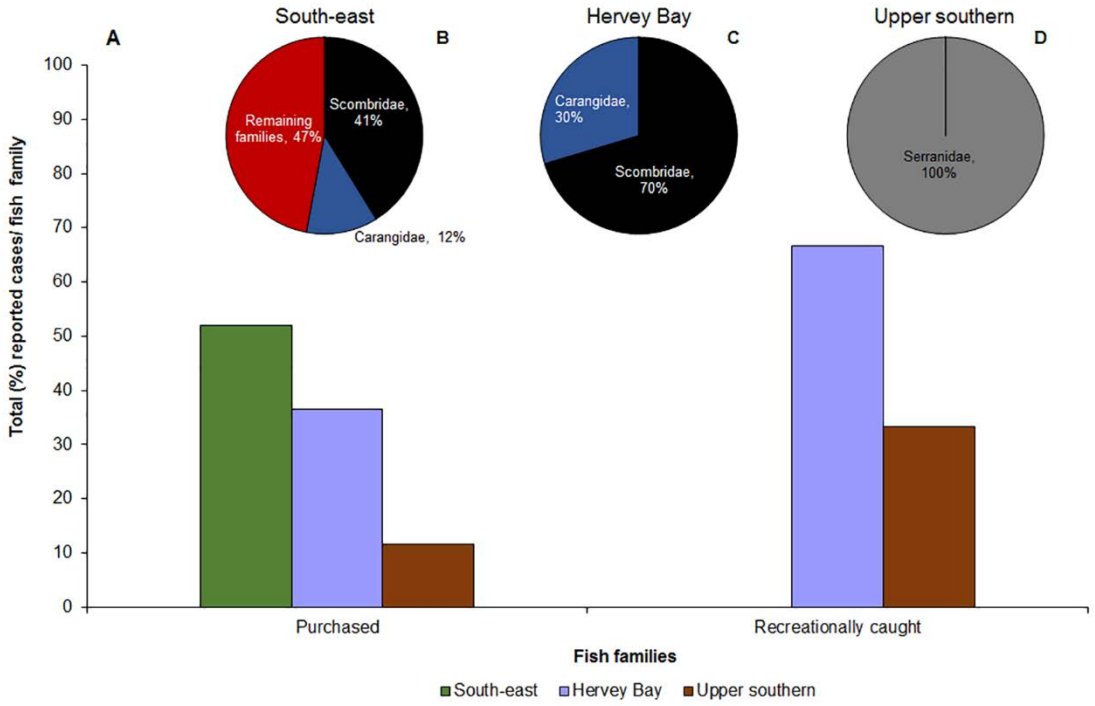

Figure 2.7 Percent of reported ciguatera cases in three southern sub-zones (see Figure 2.1) for the period 2001 – 2012: associated with purchased or recreationally caught fish (A); associated with fish families, Scombridae, Serranidae and Remaining families in the South-east area (B); Hervey Bay area (C); and Upper Southern area (D).

2.5 Discussion

Ciguatera is a tropical illness that directly impacts populations along 2,788 km of Queensland (Qld) coastline, Australia. The metropolitan south-east region supports 3.2 million people, or approximately 70% of the Qld population (Queensland-Treasury, 2015). The remaining Qld coastal population of just 1.4 million people inhabits the remaining ~ 2,500 km of Qld coastline, mostly concentrated in small to medium-sized coastal cities. Within Pacific island communities, reported ciguatera cases decrease with distance from islands that have metropolitan areas (Anderson and Lobel, 1987; Kaly et al., 1991). This decrease is a consequence of increased under-reporting of ciguatera cases

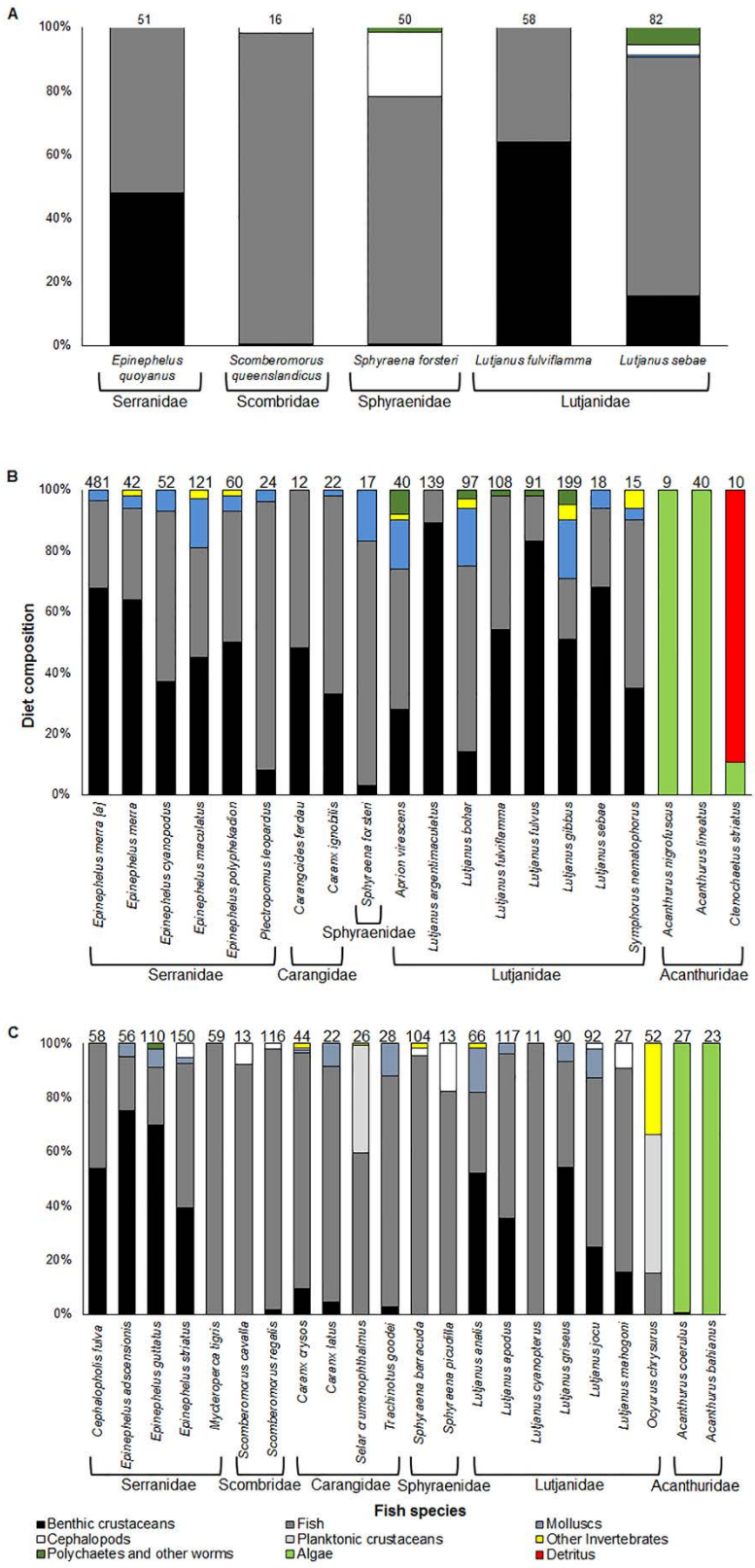

Figure 2.8 Diet composition of six fish families most frequently implicated with ciguatera (sourced from fishbase.org). These are shown for (A) Queensland; (B) the Pacific; and (C) the Caribbean. The number of fish sampled is shown above the columns. In the Pacific (B) region, the diet composition for *Epinephelus merra* was reported in more than one study and is denoted by [a].

with geographic isolation from and distance to public health facilities (Anderson and Lobel, 1987; Chateau-Degat et al., 2007; Kaly et al., 1991). This study also demonstrated that the highest number of reported ciguatera cases was closest to large cities in the south-east of Qld, with the lowest occurrence in the central and far north regions of Qld, where isolated pockets of populations occur between large uninhabited areas of National and State parks. In the 1980s, under-reporting of ciguatera cases in Qld was thought to increase with distance from the metropolitan south-east region (Gillespie et al., 1986; Gillespie et al., 1988). These studies by Gillespie and colleagues, between 1965 and 1987 included data for a possible 2,100 cases in northern Qld not reported, (Gillespie et al., 1986; Gillespie et al., 1988). Whether the regional differences in ciguatera cases observed in the present study was affected by an increased under-reporting of ciguatera with distance from the metropolitan south-east region, remains unknown. The number of ciguatera cases identified via telephone surveys in the Caribbean was 43 – 45% higher than hospital records suggested (Olsen et al., 1984; Tester et al., 2010). In Qld, a telephone survey conducted in 1985 (Capra and Cameron, 1985) estimated >50% more cases than documented in health records within the same period (Harvey, 1997). Lewis et al. (1988) suggested that extensive questionnaires are needed to provide a more accurate estimate of prevalence and distribution of ciguatera in Qld. A questionnaire is a simple method that can gather substantial quantitative and qualitative data, including lifestyle preferences, fish species availability and source of fish species in ciguatera cases. More such questionnaires will help to improve current knowledge and understanding of ciguatera. Such knowledge

can be used to develop better management practices and support the Qld public and the Qld fishing industry.

In French Polynesia, reported cases of ciguatera were more frequent during the tropical wet season compared to the dry season (Chateau-Degat et al., 2007). Similarly, in this study, a seasonal peak of ciguatera occurrence in the Austral wet season was apparent. Seasonality of ciguatera cases in Florida, USA and Puerto Rico, was associated with migration patterns of barracuda (Lawerence et al., 1980; Tosteson et al., 1988). In Qld, several mackerel species, including Spanish mackerel migrate annually into southern waters during the wet season months (Begg and Hopper, 1997), a time when monthly occurrence of ciguatera cases also peaked, particularly in December, January and March. It is likely that migration of Spanish mackerel was a driver in the high incidence of ciguatera in Qld during the Austral wet season. Chateau-Degat et al. (2005) identified a three-month lag between peak abundances of *Gambierdiscus*, the causative dinoflagellate of ciguatera, and increases in ciguatera cases in humans. Gillespie et al. (1985) observed a peak in *Gambierdiscus* abundance in September for two consecutive years on Flinders Reef, located off the south-east Qld coast. In the present study, reported cases of ciguatera were low in September, and this was the only month in which scombrids were not implicated in ciguatera. This was also approximately three months prior to monthly increases in ciguatera, which peaked in January, in association with the highest monthly occurrence of ciguatera caused by scombrids. It is hypothetically feasible that fish eaten by mackerel within south-east coastal

waters are grazing on macroalgae hosting high abundances of *Gambierdiscus* populations that occurred in September.

Exports of frozen reef fish, as well as bans and restrictions on sale of particular fish species, can also obscure seasonality of ciguatera cases (Sparrow and Heimann, 2016). Similarly, for example, a ban on the commercial sale of barracuda in the major cities of Puerto Rico resulted in a rise of ciguatera cases caused mainly by grouper, while barracuda, caught by local fishermen, continued to be the main cause of ciguatera in coastal towns (Escalona de Motta et al., 1986). Ciguatera associated with Carangidae in the present study increased to from 3% to 16% compared to previous studies between 1965 and 1995 (Gillespie et al., 1986; Harvey, 1997). At the same time, there was a 10% decline in ciguatera associated with Scombridae in the present study compared to the study by Harvey (1997) between 1976 and 1995, and by > 30% compared to the study by Gillespie et al. (1986) between 1965 and 1984. Scombrids continue to be caught recreationally in the Hervey Bay Zone 2a, a known ciguatera hotspot, and were the principal fish family implicated in ciguatera cases in this location. The decline in frequency of ciguatera caused by Scombridae and the associated rise in in cases caused by Carangidae may be a consequence of people avoiding eating scombrids from the area following a public awareness and education program conducted in 1980 (Gillespie et al., 1988). This decline may also be related to improved access to media reports on ciguatera outbreaks associated with Spanish mackerel. Further research through questionnaires could determine the effect of the education program on

public awareness and whether such knowledge was transferred to subsequent generations.

The variety of fish species locally available and dietary preferences of island residents are thought to influence which fish species are most frequently implicated in ciguatera cases in humans (Chateau-Degat et al., 2007; Lawerence et al., 1980). Serranids are often associated with ciguatera cases in tropical regions (Baumann et al., 2010; Chinain et al., 2010; Morris et al., 1982). Unlike scombrids, they are often closely associated with coral reefs. A north-south difference in the distribution of ciguatera associated with serranids and scombrids, may well be related to availability of coral reef habitat. Gillespie et al. (1986) and Harvey (1997) both noted that incidence of ciguatera associated with these two fish families seemed to shift at 23 °S. The study between 1996 and 2010 also showed a strong delineation between serranid-associated and scombrid-associated cases of ciguatera for northern and southern Qld, respectively. In this currentstudy, serranids caused 100% of ciguatera cases in the upper southern sub-zone, suggesting the shift in relative abundance from serranid- to scombrid-associated ciguatera as one moves south may extend to 25 °S. The southern end of the GBR Marine Park is located in this southern sub-zone. This may affect the availability of coral reef habitat for serranids. The habitat association for serranids with coral reefs, and the north-south migration patterns of Scombrids, likely influence local availability of these two families of fish in northern and southern Qld areas and, thereby, influence availability in the human diet.

Other factors that could influence decisions of people to eat particular types of fish include source (purchased or recreationally caught), culture, weather conditions and availability of fish. In Florida and the US Virgin Islands, purchased fish was frequently the source of ciguatera incidence (Lawerence et al., 1980; Morris et al., 1982; Stinn et al., 2000). The present study documented that purchased fish was often associated with ciguatera, particularly in southern Qld. The study further demonstrated a decreasing trend of contribution of purchased fish to ciguatera cases from southern to northern Qld. This trend may relate to the lower number of reported cases in northern Qld, greater accessibility and preference for recreational fishing per capita in northern Qld, or greater duration of suitable weather for recreational fishing. Knowledge of the sources of ciguatoxic fish can help to identify hotspots of ciguatera cases and thus improve management practices (e.g. bans, education programs).

In French Polynesia and Rarotonga, Cook Islands, where herbivorous fish are a major component of human diet, direct links between grazers and reported ciguatera cases have been observed (Chinain et al., 2010; Rongo and van Woesik, 2013). In Qld, reported ciguatera cases were caused by mesopredators, predominantly Scombridae, Serranidae and Carangidae. Fish and benthic crustaceans were identified as major prey of mesopredators frequently linked to reported ciguatera cases in Qld. Whether fish prey varies greatly among mesopredators, and whether these mesopredators target the high ciguateric risk herbivores identified by Chinain et al. (2010) and Rongo and van Woesik (2013) cannot be ascertained without further research.

In conclusion, the higher incidence of ciguatera cases in the south of Qld appears to be caused by the high population density of humans in the south-east. The spatial and temporal patterns of ciguatera incidence may also be related to migration patterns of Scombridae, particularly, Spanish mackerel. The occurrence of ciguatera in Qld was more frequent during the Austral wet season when coral reefs are often subjected to by warmer SSTs and environmental disturbances, such as cyclones, and coral bleaching. These conditions can provide new substrates (dead coral surfaces) that can be colonised by algae (both turfs and macrophytes) and thus potentially facilitate increases in the size of benthic dinoflagellate populations. This can result in a flow-on effect of increased incidence of ciguatoxins in fish, as recently documented in Rarotonga, Cook Islands (Rongo and van Woesik, 2013). Under predicted climate-change scenarios, coral reefs are likely to experience increased frequency and intensity of environmental disturbances (Cheal et al., 2017; Hoegh-Guldberg and Bruno, 2010; Hughes et al., 2003), which may further increase the risk of ciguatera in Qld. In addition, under climate change conditions, the strengthened East Australian Current (EAC) delivers warmer SSTs further south, potentially affecting a southward range expansion of *Gambierdiscus* species along the eastern Australian coastline. This would potentially bring ciguatera into the vicinity of larger human coastal populations (Booth et al., 2007; Kohli et al., 2014). Ciguatera outbreaks associated with Spanish mackerel caught in coastal waters off northern NSW were recorded between 2014 and 2016 (Farrell et al., 2016a). The effects of changing climate conditions on ciguatera distribution need to be further researched, in light of this

likely southward extension of ciguatera outbreaks into more southern geographic regions outside of Qld in Australia.

Chapter Three: The effect of the Pacific Decadal Oscillation, El Niño events and environmental disturbances on ciguatera occurrence in Queensland, Australia, from 1976 to 2012

3.1 Abstract

An increase in ciguatera incidence within the Pacific region has been linked to environmental disturbances caused by cyclones, crown-of-thorns starfish (*Acanthaster planci*) outbreaks and mass coral bleaching events. Increased incidence of ciguatera has also recently been linked to climatic variations, specifically the Pacific Decadal Oscillation (PDO) and El Niño periods. The effect of the PDO, El Niño events and environmental disturbances on ciguatera occurrence has not yet been investigated for Queensland (Qld) Australia. In this study, the Qld Health Department database for foodborne diseases was used to investigate the frequency of reported ciguatera cases in Qld between 1976 and 2012. Both long-term (~ 1.5 to 2 decades) and short term (2-5 year) variations in the frequency of ciguatera cases were detected in Qld. Rates of reported ciguatera cases in the warm phase of the PDO (mean 46 cases per year from 1977 to 1995) were two-fold higher than for the cool phase of the PDO (mean 23 cases per year from 1999 to 2003). Short-term variations of ciguatera cases in Qld appeared to be most often associated with distinct but not significant spikes in frequency a few years after El Niño events. The climatic model that explained the highest amount of variance in the occurrence of ciguatera in Qld incorporated a long-term PDO effect and a short-term effect from coral

bleaching on the Great Barrier Reef (GBR) and a short-term decline in frequency of ciguatera cases in Qld, followed by a rapid increase in frequency of ciguatera cases ~ 2 years later. Differences in the frequency of coral bleaching and cyclones occur regionally in Qld. This raises the possibility that fluctuations in ciguatera could vary regionally on the GBR. Future research on the drivers of ciguatera outbreaks in Qld requires multi-decadal, region-specific sampling of abundance of ciguatoxic, benthic dinoflagellates, co-ordinated with regional-scale questionnaires on the incidence of ciguatera in human populations.

3.2 Introduction

Ciguatera is a tropical illness caused by the consumption of tropical reef fish that have accumulated ciguatoxins through trophic transfer, primarily derived from the dinoflagellate genus, *Gambierdiscus (Argyle et al., 2016; Chinain et al., 1999b; Kohli et al., 2014; Litaker et al., 2009)*. Tourism-associated travel and consumer demand for tropical fish exports have extended the impact of ciguatera into temperate regions (Arena et al., 2004; Boada et al., 2010; Mattei et al., 2014; Wong et al., 2005). It is predicted that climate change will increase ciguatera occurrence in tropical regions and perhaps cause range expansion of *Gambierdiscus* into temperate marine habitats (Parsons et al., 2012; Tester et al., 2013). Such range expansions could result in bioaccumulation of ciguatoxins in local fish populations and flow-on effect of ciguatera outbreaks in formerly temperate regions. The first known range expansion of *Gambierdiscus* populations was into sub-tropical coastal waters off North Carolina, USA, with a subsequent record of resident fish implicated in locally reported ciguatera cases

(Morris et al., 1990). *Gambierdiscus* populations have recently expanded their range into the Canary Islands (Fraga et al., 2011), the Mediterranean (Aligizaki and Nikolaidis, 2008), Korea (Jeong et al., 2012), Japan (Nishimura et al., 2014), New Zealand (Rhodes et al., 2014) and temperate Australia (New South Wales) (Kohli et al., 2014).

In tropical regions, environmental disturbances are predicted to increase in frequency and intensity under climate change, which will likely impact coral reef health (Cheal et al., 2017; Hoegh-Guldberg and Bruno, 2010; Hughes et al., 2003). Environmental disturbances, including coral bleaching, cyclones and *Acanthaster planci* (crown-of-thorns starfish) outbreaks provide new substrata for algae (both turfs and macrophytes) to colonise, which support increases in local population sizes and also potential range expansion of their associated benthic dinoflagellate assemblages, including *Gambierdiscus* (Chinain et al., 1999b; Kohler and Kohler, 1992; Rongo and van Woesik, 2013). Increased *Gambierdiscus* populations have been recorded after coral bleaching events (Bagnis and Rougerie, 1992; Turquet et al., 2001), however, reports of subsequent increase of ciguatera cases in humans are inconsistent. The frequency of cyclones in the Caribbean (Gingold et al., 2014), as well as cyclones and *A. planci* outbreaks in the Pacific (Chinain et al., 2010; Rongo and van Woesik, 2013), have been linked to increased occurrence of ciguatera, however, little information is available on *Gambierdiscus* populations following cyclones and *A. planci* outbreaks. In Queensland (Qld), Australia, prior to the 2016 mass coral bleaching event (Hughes et al. 2017), the impact of coral bleaching on live coral cover across the Great Barrier Reef (GBR) was relatively

small, compared to the effects of *A. planci* and cyclones, which were the principal cause of coral mortality for almost three decades (Cheal et al., 2017; De'ath et al., 2012). The severity of environmental disturbances on coral reefs has varied between the northern, central and southern regions of the GBR. Prior to the 2016 coral bleaching event, there was minimal impact of coral bleaching in the northern GBR region. Cyclones were the major cause of coral loss in central and southern GBR (Cheal et al., 2017; De'ath et al., 2012), with additional loss of coral in central GBR due to *A. planci* outbreaks (De'ath et al., 2012; Sweatman et al., 2011). However, it is unknown if these environmental disturbances have been associated with increases in ciguatera at a state- or regional-scale in Qld.

Ciguatera occurrence in tropical regions has been positively associated with warmer sea surface temperatures (SSTs) (Chateau-Degat et al., 2007; Kibler et al., 2012; Litaker et al., 2009; Llewellyn, 2010), which are influenced by ocean-scale climatic systems, such as the Pacific Decadal Oscillation (PDO). Chavez et al. (2003) observed that since 1900, the PDO oscillated between a warm and cool phase approximately every 25 years, with associated large-scale fluctuations in the abundance of schooling fish species like anchovies and sardines. As ciguatera poisoning involves transfer of ciguatoxins through marine food webs to mesopredators, known to feed on schooling fish species (Begg and Hopper, 1997; Beukers-Stewart and Jones, 2004; Chinain et al., 1999b; St John et al., 2001), it is perhaps not surprising that annual reported ciguatera cases were linked to the PDO (Llewellyn, 2010), providing that similar correlated climate responses are realised by tropical species of schooling fish. Warmer SSTs during El Niño periods have also been linked to significant

increases in reported ciguatera cases for several South Pacific island nations (Hales et al., 1999; Llewellyn, 2010; Rongo and van Woesik, 2011). The onset of El Niño periods have been predicted using the Southern Oscillation Index (SOI), which is measuresf the difference in atmospheric pressure between Easter Island in the eastern Pacific and Darwin, Australia near South-East Asia (Wyrtki et al., 1976). El Niño periods have been linked to warmer than average SSTs, with the opposite phase (La Niña) associated with cooler than average SSTs. It is thought that El Niño periods occurred more frequently during the warm PDO phase, while La Niña periods have been considered more frequent in the cool PDO phase (Rongo et al., 2009).

Coral bleaching is triggered when warmer SSTs reach above the upper thermal limits of corals. Which often occurred during El Niño periods, with the frequency and severity predicted to increase with climate change. Regions of cooler and warmer SSTs were observed in the South Pacific, and appeared to align with El Niño and La Niña periods (Hales et al., 1999). Regional differences in SSTs may hence potentially affect the regional variability in ciguatera cases reported in the Pacific (Hales et al., 1999; Lewis, 1986b; Skinner et al., 2011). In Australia, ciguatera is caused from fish sourced within tropical and sub-tropical waters along the ~2,800 km Qld coastline (see Chapter 2).

In Qld, cyclones, *A. planci* outbreaks and mass coral bleaching events impact live coral cover on the Great Barrier Reef (GBR), with severity and frequency of environmental disturbances predicted to increase according to current climate

models. The role that environmental disturbances may play in ciguatera outbreaks in Qld remains unknown. While ocean-scale climatic systems such as the PDO and SOI have been linked to frequency of reported ciguatera cases in the South Pacific region, the potential impact on ciguatera frequency in Qld has not previously been investigated. The aims of this study were to investigate the potential effects of (1) climatic cycles (long-term changes in SST caused by the PDO and short-term changes in SST caused by El Niño periods); and (2) environmental disturbances (cyclones, *A. planci* outbreaks, mass coral bleaching), on the frequency of reported ciguatera cases in Qld, Australia over the period 1976 to 2012. This study is unique in that it analyses a 37-year, continual dataset on ciguatera frequency spanning over both phases of the PDO (warm and cool), including the transition between phases in the mid-1990s.

3.3 Methods

3.3.1 Study area

The eastern coast of Australia lies on the western boundary of the South Pacific Ocean. Queensland (Qld) is located on the east coast of Australia and extends from Cape York (10°S) in the north to Coolangatta (28°S) in southern Qld (Figure 3.1). The Great Barrier Reef (GBR) is over 2,000 km long, borders most of the Qld Pacific coastline and covers an area of 345,000 km^2, consisting of > 3,000 individual reefs, islands, and cays (De'ath et al., 2012). The GBR is the source of many target fish for commercial fisheries in Qld (Innes et al., 2014b; Thébaud et al., 2014). The sparsely populated coastline adjoining the far northern GBR has minimal impact on the health of the coral reef ecosystem.

Human population along the extensive Qld coastline is spatially variable. Population density is highest in south-east Qld (3.2 million people). South-east Qld extends from Noosa to Coolangatta and west to Toowoomba (Figure 3.1), and supports approximately 70% of the Qld population (Queensland-Treasury, 2015), but does not directly adjoin the GBR. The remaining population is sparsely distributed, with regional centres ranging from 67,400 people in Gladstone to approximately 200,000 people in Townsville and Cairns; and small populations outside of regional centres, such as 2,721 people in Cooktown, north of Cairns. As number of inhabitants per region influences estimated incidence rates, data are presented as the number of reported cases.

To evaluate whether any regional correlations exist between reported ciguatera cases and environmental disturbances, Qld was divided into northern, central and southern regions (Figure 3.1). Regional ciguatera occurrence was based on the location of reported cases, which was available for the period, 1996 – 2010. As coral bleaching can be triggered by warmer SSTs and low salinities, El Niño periods were included to identify differences between thermal and osmotically-induced coral bleaching.

3.3.2 Reported ciguatera cases in Queensland

In Australia, hospital and health records are collated into a database maintained by the Australian Government Department of Health. Queensland is the only Australian state where reporting of ciguatera is mandatory: other Australian

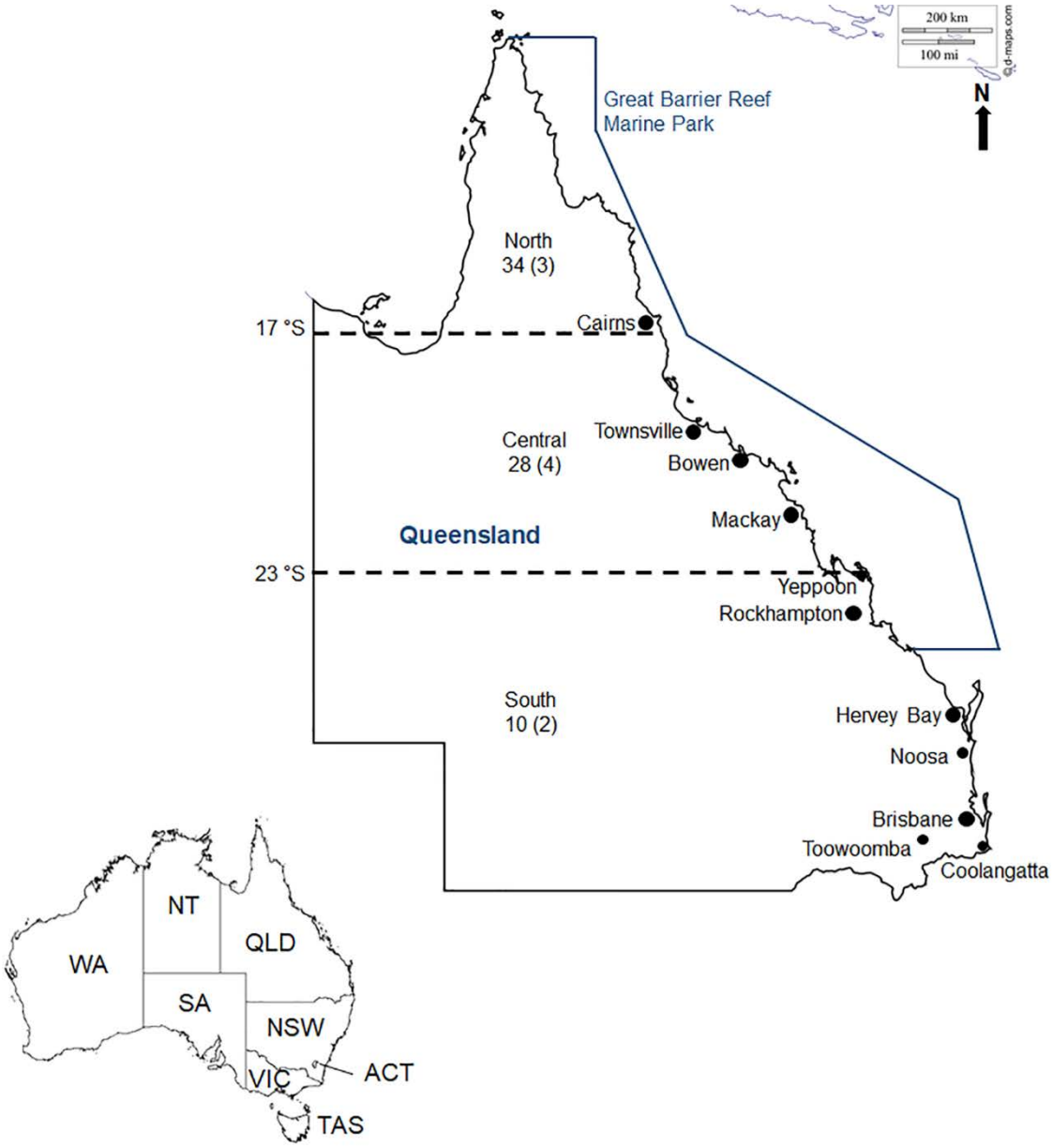

Figure 3.1 Map of Queensland (Qld), Australia, with the Great Barrier Reef (GBR) Marine Park outlined. Three Qld regions: north; central; and south with total number of cyclones (total number of severe tropical cyclones, category 4 and 5) that impacted each region between 1976 and 2102.

states record only large outbreaks and severe cases, where medical attention has been sought in hospitals (Ng and Gregory, 2000). This study utilised a publicly available database, the Ozfoodnet Working Group, which produced quarterly reports on foodborne diseases in Australia, including ciguatera, for the period 2001 - 2012. These data were supplemented with information from

Queensland Health for the period 1996 – 2000 and the work of Harvey (1997), who used available health records 1976-1995. Data prior to 1976 obtained by Gillespie et al. (1986) were excluded, as yearly occurrences of reported cases were not available, but were based on percent frequency of symptoms.

3.3.3 Climatic cycles

Yearly averages for the Pacific Decadal Oscillation (PDO) were calculated from monthly data on sea surface temperatures (SSTs) anomalies obtained from the Joint Institute for the Study of the Atmosphere and Ocean (JISAO); Washington, USA (http://jisao.washington.edu). The PDO was also categorised as negative (cool) or positive (warm), following Rongo et al. (2009). The SOI is based on pressure anomalies between Tahiti and Darwin. Monthly data from the Australian Bureau of Meteorology (www.bom.gov.au) were accessed to calculate yearly averages. As the Southern Oscillation Index (SOI) predicts periodic climatic events, including El Niño events, it was used to represent such events in the investigation of the temporal distribution of ciguatera cases in Qld. To further investigate any links between El Niño-Southern Oscillation (ENSO) cycles and reported ciguatera cases, years were also classified categorically as El Niño, La Niña, and neutral, based on which category was in effect during the Austral wet season (November – April). Categories were assigned based on the wet season as El Niño periods coincided most frequently with the wet season, as did most environmental disturbances (mass coral bleaching, cyclones, commencement of *A. planci* outbreaks).

3.3.4. Environmental disturbances

Data on environmental disturbances for the study period, 1976 – 2012 were sourced from literature and Qld governmental agencies. The occurrence of environmental disturbances and associated impacts on Qld and the GBR are summarised (Table 3.1), following a similar approach to Chinain et al. (2010) and Rongo and van Woesik (2013). Specifically, information on mass coral bleaching events was taken from the Australian Institute of Marine Science (www.aims.gov.au) and literature (Jones and Berkelmans, 2014; Oliver et al., 2009); data on cyclones that impacted the Qld coast, including the GBR were taken from the Australian Bureau of Meterology (www.bom.gov.au). A total of 63 cyclones impacted the Qld coast over the 37-year study period, with 55 cyclones being ≤ category three. There was no observable trend due to the high frequency of low-impact cyclones (≤ category three), therefore, only observations for severe cyclones (category four and five) are described.

Data for *A. planci* outbreaks were sourced from the literature, which recorded four outbreaks on the GBR; 1962 -1976, 1979 – 1991, 1993 - 2005, and 2009 – current (Figure 3.2). There was an absence of a discernible effect between *A. planci* outbreaks and yearly reported ciguatera cases in Qld due to: the average 14-year duration of outbreaks; multiple outbreaks during the study period; and because north and southwards migration of outbreaks across the GBR could not be regionally evaluated given the paucity of yearly monitoring data.

Table 3.1 A summary of natural disturbances (cyclones, mass coral bleaching and crown-of-thorns starfish (*Acanthaster planci*) outbreaks) as well as their impact on Queensland (Qld) coastal populations and the Great Barrier Reef (GBR), between 1976 and 2012. Cyclone data (including point of impact on the coast, wind data, damage and flooding) and cyclone severity based on category, taken from the Australian Bureau of Meteorology (www.bom.gov.au). Records for *A. planci* outbreaks were taken from Moran et al. (1988), De'ath et al. (2012) and Wooldridge and Brodie (2015). Information on coral bleaching events was taken from the Australian Institute of Marine Science (www.aims.gov.au), Jones and Berkelmans (2014) and Oliver et al. (2009).

Year	Environmental disturbance	Cyclone severity	Description of impact
1976	*A. planci* outbreak ends;		*Acanthaster planci* outbreak (1962 – 1976), commenced at Green Island, off Cairns and moved southwards[c].
	Cyclones: David	3	*David*: hit north of St. Lawrence, wind speeds up to 175 km/h and wave height up to 8.9m. Extensive damage to Heron Is., 30 homes unroofed in Yeppoon and flooding extended down to Moreton Bay, Brisbane.
	Alan	2	*Alan*: hit near Bloomfield River Mission, caused widespread flooding inland.
	Beth	3	*Beth*: hit near Bundaberg, 200 homes unroofed, two aircraft damaged and flash flooding in Maryborough-Bundaberg area.
	Dawn	1	*Dawn*: travelled off the coast, north to south, crossed Fraser Island. Two homes unroofed in North Mackay, uprooted trees on Heron Island and flash flooding in Proserpine and Bundaberg.
1977	Cyclones: Keith	1	*Keith*: first hit east of Cairns and then at Cape Cleveland, south of Townsville. Caused extensive damage to tropical rainforest over the Graham Range, wind and rain caused extensive loss to banana and sugar crops. Two deaths.
	Otto	2	*Otto*: formed in the Gulf of Carpentaria, moved into the Coral Sea near Cape Tribulation before and hit again near Bowen, wave height up to 6.3m. Worsened existing flooding between Cairns and Ingham, caused $6 million (at 1977) crop and property damage, and destroyed 1200 m of esplanade at Cairns.
1978	Cyclone: Hal	3	*Hal*: formed in the Gulf of Carpentaria and crossed land into the Coral Sea north of Cape Tribulation, wind speeds up to 140 km/h. No structural damage.
1979	*A. planci* outbreak started;		*Acanthaster planci* outbreak reported on reefs in the Cairns area from 1979 to 1982[a], commenced at Green Island, off Cairns; moved southwards into central Great Barrier Reef (GBR) 560 km, northward movement into north GBR 150 km ended 1984[b]
	Cyclones: Peter	2	*Peter*: formed in the Gulf of Carpentaria and crossed land into the Coral Sea near Cooktown. Mt Bellenden Ker, 70 km south of Cairns recorded 1140 mm in 24 hours, caused flooding from

Year	Environmental disturbance	Cyclone severity	Description of impact
			Cooktown to Tully, estimated $10 million (at 1979) damage and two deaths.
	Greta	2	*Greta*: formed in the Gulf of Carpentaria and crossed land in the Cape York Peninsula, hit again at Princess Charlotte Bay. Flooding extended south of Cairns, no significant damage reported.
	Gordon	1	*Gordon*: impacted Frederick Reef in the Coral Sea, winds up to 83 km/h. Weakened to a tropical low and hit south of Proserpine, caused some beach erosion.
	Severe cyclone: Kerry	4	*Kerry*: hit across the Whitsunday Islands, winds up to 252 km/h recorded over the Coral Sea, heavy rainfalls but no damage or flooding.
1980	Mass coral bleaching; *A. planci* outbreak active;		Mass coral bleaching event minor, up to 10% coral cover bleached.
	Cyclones: Paul	2	*Paul*: formed as a tropical low in the Gulf of Carpentaria and moved southward overland, caused severe flooding down the Don River through Bowen, destroyed two homes and several million dollars (at 1980) damage to the market garden industry. Entered the Coral Sea south of Mackay, near Sarina, upgraded to a cyclone northeast of Yeppoon, continued a southeast path away from the coast.
	Ruth	2	*Ruth*: formed in the Coral Sea and did not cross the coast, but concurrent extreme high tides resulted in rough seas and heavy swells, caused extensive beach erosion in southeast Qld.
	Severe cyclone: Simon	4	*Simon*: hit north of Yeppoon then turned back to sea and again hit at Sandy Cape, Fraser Island, winds up to 140 km/h, caused erosion to shorelines from Yeppoon to Bundaberg, minor structural damage at Gladstone, yacht blown ashore at Lady Elliot Island and a RAAF helicopter crashed on Fraser Island, no deaths.
1981	*A. planci* outbreak active;		
	Cyclones: Eddie	3	*Eddie*: hit Princess Charlotte Bay, caused minor flooding in north-eastern coastal rivers.
	Cliff	2	*Cliff*: hit at Bundaberg, winds up to 110 km/h, caused wind damage to cane crops and several homes. Minor flooding, beach erosion from Bundaberg to Coolangatta, southeast Qld and one death.
	Freda	3	*Freda*: Formed in the Gulf of Carpentaria crossed land, caused minor flooding in northern coastal rivers. Upgraded to a cyclone after it crossed into the Coral Sea near Cooktown, moved 380 km offshore and caused gales on offshore islands, capsized a 10 m fishing trawler but no deaths or significant damage.
1982	Mass coral		*A. planci* outbreaks reported on reefs in the

Year	Environmental disturbance	Cyclone severity	Description of impact
	bleaching; *A. planci* outbreak active;		Princess Charlotte Bay area from 1982 to 1984[a]. Mass coral bleaching event heavy, above 50% coral cover bleached.
	Cyclone: Dominic	1	*Dominic*: formed in the Gulf of Carpentaria, weakened to a tropical low as it crossed the Cape York Peninsula into the Coral Sea. Hit again north of Cooktown, no structural damage or flooding.
1983	*A. planci* outbreak active; Severe cyclone: Elinor	4	*Elinor*: weakened as it moved towards the coast and hit near Carmila, south of Mackay. Wind speed up to 128 km/h at Marion Reef, northeast of Mackay, caused minor damage to the central coastline and adjacent islands.
1984	*A. planci* outbreak active;		*Grace*: formed in the Coral Sea northeast of Townsville and moved south passing near Willis
	Cyclones: Grace	3	Island, Lihou and Frederick Reefs, Cato Island without crossing the coast, caused minor beach erosion in southeast Qld.
	Ingrid	1	*Ingrid*: formed approximately 100 km south of Willis Island, wind speed up to 111 km/h and caused minor flooding between Cairns and Mackay.
	Jim	1	*Jim*: hit near Cape Grenville, moved west across land and entered the Gulf of Carpentaria, no damage reported on the east coast.
	Kathy	1	*Kathy*: hit near Pascoe River, Cape York, no damage reported. Moved across land into the Gulf of Carpentaria, three fishing trawlers forced aground, one death and numerous dugongs and green sea turtles carried inland up to 8km.
1985	*A. planci* outbreak active;		*A. planci* outbreak status surveyed March 1985 to January 1986[a]: active outbreaks on 17 reefs in central GBR with live coral cover at low levels (1 - 10%). A total of 3395 *A. planci* recorded on 19 reefs; off Mackay active outbreaks on 4 reefs with moderate to severe coral mortality; 169 *A. planci* recorded on 10 reefs. Live coral cover at moderate to high levels (10 - 50%) in central GBR (on reefs not impacted by outbreaks) and north GBR (except between Cooktown and Lizard Island, live coral cover was low to moderate, 1 - 30%, with algal covered coral observed on many reefs). No recent *A. planci* outbreaks observed in south GBR or in far north GBR, in the Cape Grenville area (extensive algal covered coral observed on 2 reefs that had no prior evidence of *A. planci* outbreaks).
	Cyclones: Pierre	1	*Pierre*: formed approximately 160 km east of Cooktown, moved south and hit in Shoalwater Bay, north of Yeppoon, caused minor damage and flooding. Wind speed up to 102 km/h at Hayman Island, Whitsundays region.
	Tanya	2	*Tanya*: hit in Princess Charlotte Bay, Cape York Peninsula, wind speed up to 93 km/h, caused minor damage to vegetation.

Year	Environmental disturbance	Cyclone severity	Description of impact
1986	*A. planci* outbreak active;		
	Cyclones:		
	Winifred	2	*Winifred*: formed approximately 450 km north of Cairns and hit south of Innisfail, wind speed up to 176 km/h, caused damage up to $150 million (at 1986), including crop losses estimated at $90 million and three deaths.
	Manu	1	*Manu*: hit at Cooktown, wind speeds up to 74 km/h, no damage reported.
1987	Mass coral bleaching; *A. planci* outbreak active		Mass coral bleaching event moderate, 10 - 50% coral cover bleached.
1988	*A. planci* outbreak active;		
	Cyclone:		
	Charlie	2	*Charlie*: hit at Upstart Bay, south of Ayr, wind speed up to 115 km/h, caused minor structural damage, moderate damage to sugar cane crops.
1989	*A. planci* outbreak active;		
	Cyclones:		
	Aivu	3	*Aivu*: hit in the Burdekin River delta, near Home Hill, wind speed up to 118 km/h at Holmes Reef, about 240 km east, northeast of Cairns. Damage estimated at $90 million (at 1989), caused severe flooding between Townsville and Mackay, destroyed several beachfront properties, and one death.
	Meena	1	*Meena*: hit in the Cape York Peninsula, wind speed up to 93 km/h, no damage reported.
1990	*A. planci* outbreak active;		
	Cyclones:		
	Nancy	1	*Nancy*: off the coast at Brisbane, wind speeds up to 100 km/h, caused flash flooding south of Brisbane and four deaths.
	Ivor	2	*Ivor*: hit the Cape York Peninsula, moved westward into the Gulf of Carpentaria and re-crossed the Peninsula north of Cairns, again hit south of Townsville and then near Mackay. Wind speed up to 125 km/h, caused minor damage and severe flooding.
	Joy	2	*Joy*: hit at Townsville, wind speed up to 167 km/h, severe damages in Port Douglas and in the Mackay region; also, resulted in flooding from the Fitzroy River in southern Qld, freshwater plume lasting approximately 13 days, caused 30 - 90% coral mortality at inshore Keppel reefs up to 2.3 m depth. Keppel reefs recovered after 10-15 years[e].
1991	*A. planci* outbreak ended		*A. planci* outbreak (1979 - 1991), commenced at Green Island, off Cairns and moved southwards into central GBR 560 km by end of outbreak in 1991; second set moved northward into north GBR 150 km, ended 1984. Southward outbreak followed similar path to outbreak from 1962-1976, 35 reefs impacted by both outbreaks with an average time of 14.68 years between outbreaks[b].
1992	Mass coral		Mass coral bleaching event light, up to 10% coral

Year	Environmental disturbance	Cyclone severity	Description of impact
	bleaching;		cover bleached.
	Cyclones:	2	*Fran*: hit near the Town of Seventeen Seventy,
	Fran		passed over Fraser Island after it turned back to Coral Sea. Crop losses in Bundaberg, flooding and minor damage in southeast Qld estimated at $2.5 million (at 1992).
	Nina	1	*Nina*: formed in the Gulf of Carpentaria, crossed the Cape York Peninsula into the Coral Sea and continued to the Solomon Islands. No damage reported.
1993	*A. planci* outbreak started		
1994	Mass coral bleaching; *A. planci* outbreak active;		Mass coral bleaching event light, up to 10% coral cover bleached.
	Cyclone:		*Rewa*: Erratic path in the Pacific, approached Qld
	Rewa		coast just south of Mackay and tracked south parallel to the Queensland coast. Heavy rain in
		2	Brisbane, one death.
1995	*A. planci* outbreak active		
1996	*A. planci* outbreak active;		*Celeste*: formed north of Townsville, moved close
	Cyclones:	2	to Bowen then moved seaward. 18 houses
	Celeste		damaged in Bowen.
	Dennis	1	*Dennis*: formed in the Gulf of Carpentaria, crossed the Cape York Peninsula into the Coral Sea, no damage reported.
	Ethel	1	*Ethel*: formed in the Gulf of Carpentaria, crossed the Cape York Peninsula into the Coral Sea at Cape Grenville, hit again near Cape Melville and back to the Gulf of Carpentaria, minor damage to vegetation.
1997	*A. planci* outbreak active;		
	Cyclones:	1	*Gillian*: weakened to a tropical low before it hit
	Gillian		near Townsville, no reported damage.
	Ita	1	*Ita*: hit southeast of Townsville, minor damage and flooding.
	Justin	2	*Justin*: hit northwest of Cairns, returned to sea north of Townsville, caused damage between Cairns and Townsville, two deaths, estimated $150 million (at 1997) loss to agricultural industry.
1998	Mass coral bleaching; *A. planci* outbreak active;		Mass coral bleaching event heavy, above 50% coral cover bleached, impacted 21% offshore and 74% inshore reefs from 654 reefs surveyed. Good recovery, < 5% of reefs suffered high mortality of corals, except in the Palm Island area, up to 72% mortality.
	Cyclone:	1	*Nathan*: tracked southeast parallel to northern
	Nathan		Cape York Peninsula coast, moved out to sea, no

Year	Environmental disturbance	Cyclone severity	Description of impact
			damage reported.
	Severe cyclone: Katrina	4	*Katrina*: Erratic path in the Coral Sea, within 500 km off the coast at Cairns and tracked southeast parallel to coast, returned to Coral Sea north of Rockhampton, no damage reported on Qld coast.
1999	*A. planci* outbreak active; Cyclone: Rona	2	*Rona*: hit north of Cow Bay, Cape York Peninsula, caused major damage between Cape Kimberley and Cape Tribulation, flooding between Cairns and Townsville, wave height above 6.3m at Low Isles, northeast of Cairns.
2000	*A. planci* outbreak active;		
	Cyclones: Steve	2	*Steve*: hit north of Cairns and moved overland towards the Gulf of Carpentaria, no report of damage.
	Tessi	2	*Tessi*: hit 75 km northwest of Townsville, caused minor damage and landslide.
	Vaughan	2	*Vaughan*: west of coast, north of Cairns but did not hit, caused heavy rainfall.
2001	*A. planci* outbreak active;		
	Cyclone: Abigail	1	*Abigail*: hit north of Cairns, minor flooding and some damage.
2002	Mass coral bleaching; *A. planci* outbreak active		Mass coral bleaching event heavy, above 50% coral cover bleached, 54% of 641 reefs surveyed, impacted nearly 41% offshore, 72% inshore reefs. Good recovery, < 5% of reefs suffered high mortality of corals, except in the Bowen area, up to 70% mortality.
2003	*A. planci* outbreak active;		
	Cyclone: Erica	1	*Erica*: low moved off the coast near the Whitsunday Islands, caused some damage in Cairns as moved offshore and upgraded to a cyclone in the Coral Sea.
2004	*A. planci* outbreak active;		
	Cyclone: Fritz	1	*Fritz*: hit near Cape Melville, no damage reported and cyclone continued over land to the Gulf of Carpentaria.
2005	*A. planci* outbreak ended;		*A. planci* outbreak (1993 - 2005), commenced at Michaelmas Cay, movement north and south in the GBR.
	Severe cyclone: Ingrid	5	*Ingrid*: hit south of Lockhart River, Cape York Peninsula, no damage reported, cyclone continued over land to the Gulf of Carpentaria.
2006	Mass coral bleaching;		Mass coral bleaching event moderate, 10 - 50% coral cover bleached, heavy in south GBR reefs,

Year	Environmental disturbance	Cyclone severity	Description of impact
			especially the Keppel Islands with up to 98% corals bleached, caused nearly 39% mortality of corals on reef flats, 32% on reef slopes.
	Cyclones: Jim	1	*Jim*: formed 370 km east of Innisfail and moved into the Coral Sea, caused flooding in the north with gale force winds as far south as the Whitsunday Islands and at Lihou Reef, Coral Sea.
	Larry	3	*Larry*: hit near Innisfail, caused flooding and extensive damage to infrastructure, crops and approximately 10, 000 homes. Damage estimated above $500 million (at 2006).
	Monica	3	*Monica*: hit south of Lockhart River, Cape York Peninsula with no damage reported, cyclone continued over land to the Gulf of Carpentaria.
2007			**No environmental disturbances recorded**
2008	Mass coral bleaching		Heavy rain caused flooding, resulted in freshwater influx and osmotic-induced mass coral bleaching event
2009	*A. planci* outbreak started;		
	Cyclone: Ellie	1	*Ellie*: hit north of Cardwell, wind speed up to 75 km/h, caused flooding between Cardwell and Bowen.
	Severe cyclone: Hamish	5	*Hamish*: formed 300km east of Princess Charlotte Bay and tracked south-southeasterly parallel to the coast, wind speed up to 215 km/h. The Whitsunday Islands, Heron, Lady Elliot and Fraser Islands were evacuated, fishing trawler capsized in the Swains Reef, caused severe damage to over 500 km of coral reefs in central and southern GBR, live coral reduced to 10% on some reefs and macroalgal blooms reported, bulk carrier off Stradbroke Island damaged, caused oil slick on southeast Qld beaches.
2010	*A. planci* outbreak active;		
	Cyclones: Olga	1	*Olga*: downgraded to a tropical low and hit the coast 80 km south of Cairns, no damage reported.
	Ului	3	*Ului*: crossed the Whitsunday Islands region and hit near Airlie Beach, wind speed up to 215 km/h, caused damage to trees, sugarcane crops, structural damage and numerous boats damaged or destroyed.
2011	Mass coral bleaching; *A. planci* outbreak active;		Mass coral bleaching event due to freshwater influx, included flooding in south Qld from the Fitzroy River with freshwater plumes lasting up to 18 days; caused almost 100% loss of live coral cover up to 8m depth on inshore Keppel reefs[e].
	Cyclones: Zelia	2	*Zelia*: formed approximately 750 km offshore, northeast of Cairns, tracked southeasterly towards Norfolk Island and remained in the Coral Sea, no

Year	Environmental disturbance	Cyclone severity	Description of impact
			damage reported.
	Anthony	2	*Anthony*: hit near Bowen, wind speed up to 95km/h, no damage reported.
	Severe cylone: Yasi	5	*Yasi*: hit near Mission Beach, one of most powerful cyclones recorded to affect Qld, communities in direct line and low lying areas were evacuated, caused damage to communities from Cairns to Proserpine, banana and sugarcane crop losses, property, infrastructure and tree damage, 150 homes destroyed, one death. Damage estimated at $3.5 billion (at 2011).
2012	*A. planci* outbreak active;		*A. planci* outbreak (2009 - current), initiation zone between Cairns and Cooktown, movement southwards. Between 1985 and 2012 live coral cover over the GBR declined from 28% to 13.8%; loss of live coral cover attributed to: 48% by tropical cyclones; 42% by *A. planci* outbreaks; and 10% by coral bleaching. Cyclones and *A. planci* outbreaks severely impacted live coral cover in southern GBR; far north GBR recorded increase in live coral cover with least impact from disturbances. Majority of loss in live coral cover in the GBR occurred since 1998[d].
	Cyclone: Jasmine	1	*Jasmine*: tropical low moved off Cape York Peninsula, caused minor tree damage and heavy rains before upgraded to a cyclone and continued easterly into the Coral Sea.

[a] (Moran et al., 1988); [b] (Moran et al., 1992); [c] (Brodie and Waterhouse, 2012); [d] (De'ath et al., 2012); [e] (Jones and Berkelmans, 2014)

3.3.5 Time lag series

To investigate links between reported ciguatera cases and environmental disturbances or ocean-scale climatic systems, a time lag of one and two years was applied based on the time lag (16 – 20 months) identified by Chateau-Degat et al. (2005), and based on yearly reporting of ciguatera cases (Llewellyn, 2010; Rongo and van Woesik, 2011).

3.3.6 Data analysis

To test for a link and potential interactions between climate and ciguatera, generalised linear models (GLMs) were used, including SOI, PDO, and potential interactions. An intercept-only model with no effects was also tested. The response variable was modelled with a negative binomial error structure, using the R function “glm.nb” in library MASS (Venables and Ripley, 2002), after tests of residuals of Poisson GLMs yielded highly significant evidence of over-dispersion. The model set included models with zero lag time (ciguatera cases in each year were modelled as a function of climate variables in the same year), a lag time of one year (ciguatera cases in each year were modelled as function of climate variables in the preceding year), and a lag time of two years. The models considered both categorical and ordinal climate measures (categorical El Niño/La Niña with PDO phase, categorical El Niño/La Niña with yearly average PDO index, yearly average SOI with PDO phase, and yearly average SOI with yearly average PDO index). This yielded a total of 37 models (Supplementary Figure S3.1, Appendix D). For each nested subset of models (for instance, all models including categorical SOI and PDO at lag zero are nested within the model in which those two variables have main and interaction effects), likelihood ratio tests were used to find the best-from-subset model, using the threshold of $P<0.05$ as the standard to favour a more complex model over a simpler one. Then, because these best-from-subset models are not nested with respect to each other (for instance, the best model for models using categorical SOI and PDO variables at lag zero are not nested within the models using the same variables at lag one, nor are they nested within models using ordinal SOI or PDO variables), The Akaike Information Criterion (AIC) was used

to compare them with each other to obtain an overall best model. For this second analysis, Akaike weights were used to estimate model selection uncertainty: the Akaike weight of a model is an estimate of the probability that that model is actually the best one among the set being compared.

To test for an additional effect of coral bleaching, over and above climatic effects, the best ciguatera frequency model from the above analyses was used to assess whether including bleaching (lag zero, one, or two years, and with and without interactions with climatic variables) yielded a better model for ciguatera frequency. As in the previous analysis, AIC was used to compare these models with one another, since the alternative bleaching models (due to their different time lags) were not nested.

The goodness-of-fit of the best-fitting model was tested in various ways. In addition to standard linear model diagnostics (residuals versus predicted values, versus explanatory variables, versus time, etc.), over-dispersion was tested by comparing the model deviance with a chi-squared distribution (as per the preliminary analysis with Poisson error structures). Temporal autocorrelation was also tested by examining whether there was significant autocorrelation in model residuals at any time lag, using the R function acf (R-Core-Team., 2015).

3.4 Results

3.4.1 Climatic systems

For all model subsets that included PDO phase (positive or negative), the best-from-subset model was always the model with an effect of PDO phase only (Supplementary Figure S3.1). For all model subsets that included PDO index instead of PDO phase, the best-from-subset model was always the model with no effects of either PDO or SOI (i.e., the intercept-only model: Supplementary Figure S3.1). Thus, there were only four distinct best-from-subset models to compare: PDO phase at lag – zero, PDO phase at lag – one, PDO phase at lag – two, and intercept-only. Model selection by AIC indicated that, of these four models, the PDO phase models had comparable levels of support for all time lags (Table 3.2), with the PDO phase at lag – two the estimated best model with 54% support, followed by the PDO phase at lag – zero, with 33% support. The intercept-only model had only 3% support.

Table 3.2 Model selection comparing the best-from-subset models (Supplementary Figure S3.1) with one another. AIC is Akaike's Information Criterion; ΔAIC is the difference between the AIC of the indicated model and that of the estimated best model (i.e., the estimated best model, by definition, has ΔAIC=0); and w_{AIC} is the Akaike weight: a measure of the probability that the indicated model is the true best model for the system.

Model	AIC	ΔAIC	w_{AIC}
PDO phase only (lag 2)	325.5	0	0.54
PDO phase only (lag 0)	326.5	1.01	0.33
PDO phase only (lag 1)	328.7	3.21	0.11
Constant (intercept only)	331.4	5.9	0.03

For the three PDO-phase models, the estimate of the effect of PDO was similar, with the number of ciguatera cases estimated to be 85% higher during the positive than the negative PDO phase for the (estimated best-fitting) PDO phase at lag – two model; 78% higher for the PDO phase at lag – zero model, and 62% higher for the PDO phase at lag – one model. As PDO phase at lag – two was the best climate-based model, it was selected to compare models incorporating coral bleaching history within the study period.

Table 3.3 Model selection comparing the best climate-based model with alternatives that incorporate bleaching history. AIC is Akaike's Information Criterion; ΔAIC is the difference between the AIC of the indicated model and that of the estimated best model (i.e., the estimated best model, by definition, has ΔAIC=0); and w_{AIC} is the Akaike weight: a measure of the probability that the indicated model is the true best model for the system.

Model	AIC	ΔAIC	w_{AIC}
PDO phase (lag 2) + Bleaching (lag 0)	322.0	0	0.54
PDO phase (lag 2) x Bleaching (lag 0)	324.0	2.0	0.20
PDO phase (lag 2)	325.5	3.5	0.09
PDO phase (lag 2) + Bleaching (lag 1)	327.1	5.1	0.04
PDO phase (lag 2) + Bleaching (lag 2)	327.4	5.4	0.04
PDO phase (lag 2) x Bleaching (lag 2)	327.5	5.5	0.03
Bleaching (lag 0)	327.5	5.5	0.03
PDO phase (lag 2) x Bleaching (lag 1)	329.0	7.0	0.02
Constant (intercept only)	331.4	9.4	<0.01
Bleaching (lag 1)	332.7	10.7	<0.01
Bleaching (lag 3)	333.4	11.4	<0.01

Model selection by AIC indicated that, of all possible models including PDO phase at lag – two and bleaching, the best model had an effect of PDO phase at lag – two, with bleaching at lag – zero, and no interaction (Table 3.3). This model had 54% support. The second-best model (with 20% support) had the same explanatory variables, but included an interaction. The third best model was the PDO phase at lag – two from the first analysis (9% support). All other models had <5% support. The effect of coral bleaching on ciguatera cases was negative, with ciguatera frequency being 40% less prevalent during coral bleaching years than non-bleaching years.

A χ^2 test against the model deviance indicated that the PDO-phase models and the PDO-bleaching models all fit adequately (P>0.29 and P=0.27, respectively). Moreover, there was no statistically significant temporal autocorrelation in the residuals, at any lags, indicating that the explanatory variables adequately accounted for the temporal structure in the data.

3.4.2 Environmental disturbances impacting Queensland coast and reefs from 1976 to 2012

Queensland (Qld) coast and reefs were impacted by disturbances in all years over the 37-year period, except for 2007 (Table 3.1). In the 37-year study period, the number of cyclones decreased southwards from the north region, but a higher number of severe tropical cyclones impacted the coast in the central region (Figure 3.1). Seven severe cyclones (category four and five) impacted the Qld coast with category five cyclones only recorded since 2005 (Table 3.1). Coral bleaching was triggered by warmer than average SSTs

associated with El Niño periods, except in 1980 (no reliable records on the cause) and in 2008-2009 and 2010-2011, when flooding and mass coral bleaching induced by low salinity was recorded (Figure 3.2).

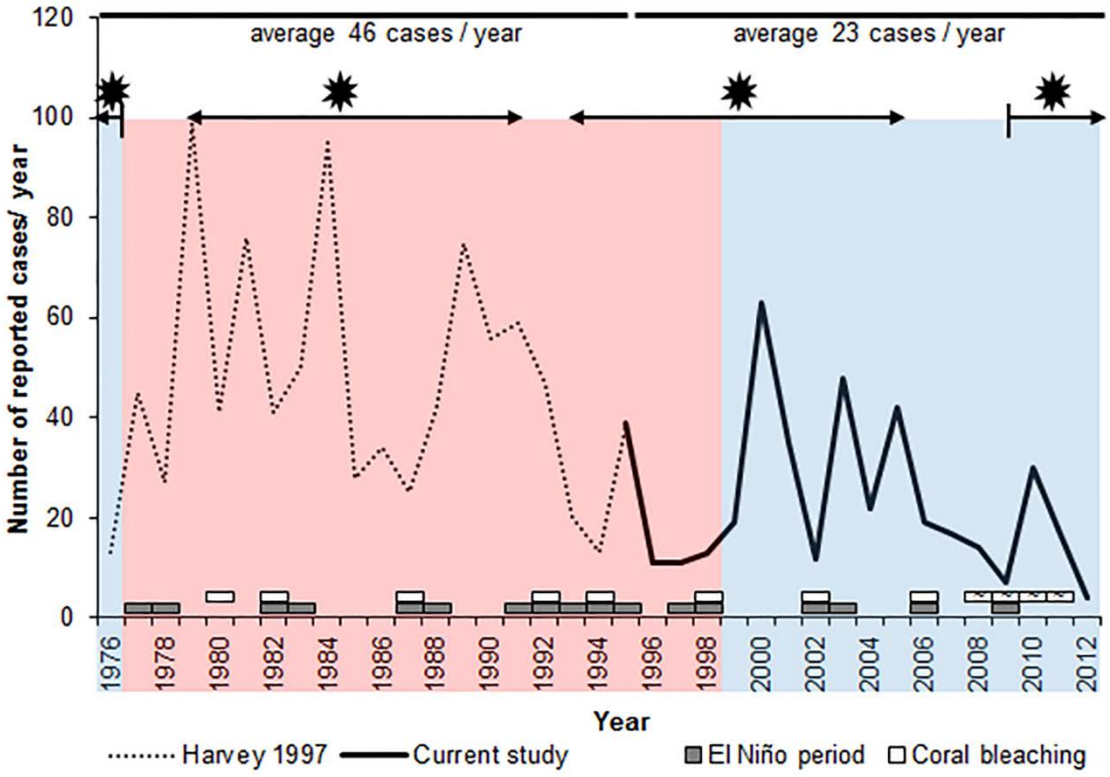

Figure 3.2 The frequency of reported cases of ciguatera incidence in Queensland (Qld), Australia over a 37-year period, 1976 – 2012 (combined with Harvey 1997). Reported cases/ year are shown for the 20-year study of Harvey 1997 and the 17-year period of the current study. Star shapes and arrows indicate *Acanthaster planci* outbreaks. The two oscillations of the PDO are shown by a blue shaded area for the cool phase (1976; 1999-2003) and a red area (1977 – 1995) to indicate a warm phase. Coral bleaching due to warm water events indicated by white rectangles and coral bleaching due to freshwater influx indicated by ~. El Niño years obtained from the Australian Government Bureau of Meteorology (www.bom.gov.au).

3.4.3 Ciguatera occurrence at a regional-scale

Environmental disturbances often impact at a regional-scale and therefore ciguatera occurrence may vary by region. In the period between 1996 and 2010, the frequency and type of environmental disturbance differed between the three Qld regions (Figure 3.3). Coral bleaching and a severe cyclone in 1998

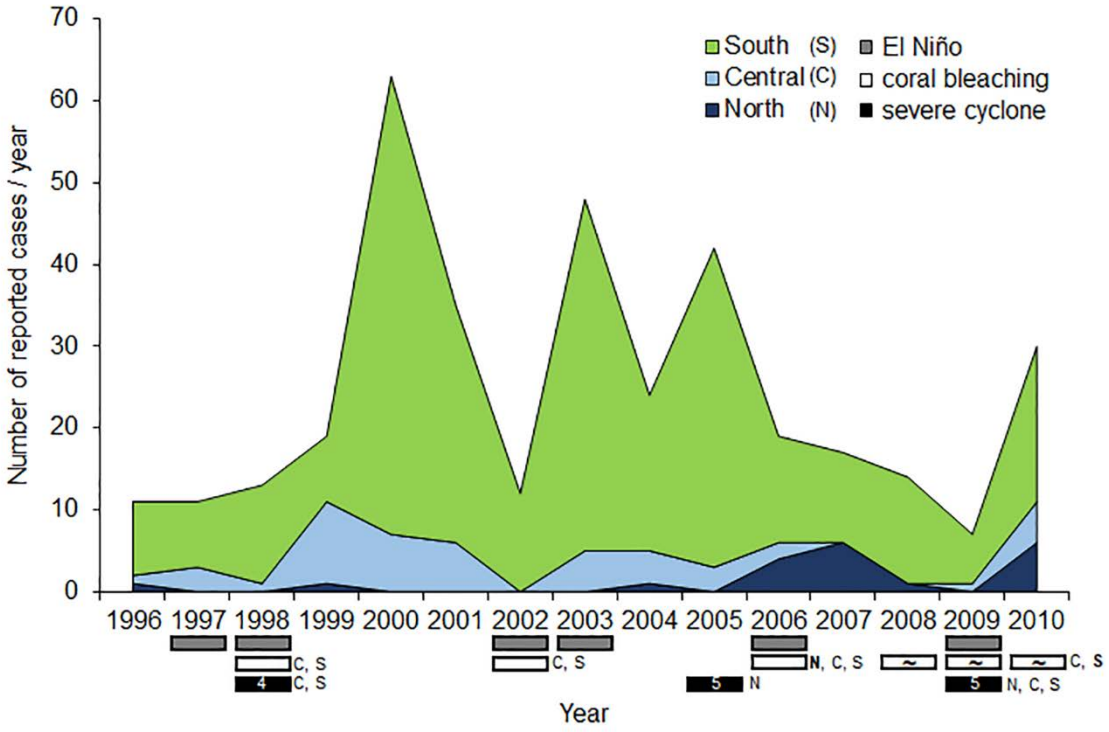

Figure 3.3 Reported ciguatera cases in the north, central and southern regions of Queensland (Qld), Australia 1996 – 2010 and impact of environmental disturbances: El Niño; coral bleaching (coral bleaching due to freshwater influx indicated by ~); severe cyclones (category indicated by number).

affected the central and southern regions of the GBR, with an increase in ciguatera occurrence observed one and two years after, respectively (Figure 3.3). Similarly, in 2002, an increase in ciguatera occurrence was observed one year after coral bleaching affected central and southern regions. Coral bleaching was not recorded for the northern region until 2006, following a severe cyclone the previous year. The occurrence of ciguatera in the north increased in the year following the cyclone and again after coral bleaching in 2006 (Figure 3.3). By contrast, the central and southern regions were less affected by coral bleaching in 2006, and a decline in ciguatera incidence was observed in the following years. A severe cyclone affected all regions of the GBR in 2009, with an increase in ciguatera occurrence observed in each region the following year. Heavy seasonal rainfalls between 2008 and 2010 also

triggered low salinity-induced coral bleaching in the central and southern regions (Figure 3.3). It is noted that although reported ciguatera cases increased generally the year after a cyclone impacted a region, it frequently coincided with other environmental disturbances, and therefore, the individual effects of cyclones and other environmental disturbances are often difficult to identify.

3.5 Discussion

Many factors contribute to fluctuations in the reported occurrence of ciguatera, including extent of public awareness, medical treatment sought and reported, government regulations and fish migrational patterns (Sparrow and Heimann, 2016). Climatic variations like the PDO, SOI, and El Nino and environmental disturbances like cyclones, coral bleaching events, however, appear to have an over-riding influence on frequency of ciguatera cases in Qld. The unique 37-year study has demonstrated a significantly higher occurrence of reported ciguatera cases in Qld during the PDO warm phase (1977 – 1998). Assuming a typical 20 – 25 year period for each PDO phase, the transition from the current cool phase may occur in the next six years (2018-2023) or may even be starting now, in 2017. The results thus suggest that the occurrence of reported cases of ciguatera in Qld is likely to increase soon, particularly during 2018-2023. Indeed, an increase of reported ciguatera cases in 2013 and 2014 in Qld (OzFoodNet-Working-Group, 2013a, 2013b, 2014a, 2014b, 2014c), followed by the third global mass coral bleaching event in 2015-2016 (Hughes et al., 2017), suggests strongly that a shift between PDO phases from cool to warm may be imminent.

3.5.1 Influence of climatic variations on ciguatera

The PDO, SOI and El Niño are climatic variations that can produce ocean-scale temperatures warmer than average SSTs in the Pacific Ocean, and have been linked to increased ciguatera incidence (Hales et al., 1999; Llewellyn, 2010; Rongo et al., 2009). In the period from 1973 to 1996 (~the warm PDO phase -1977 – 1998), several Pacific islands had a significant relationship between annual ciguatera incidence and the PDO, at different lag times from zero to two years (Llewellyn, 2010). While positive relationships were observed between annual reported ciguatera cases and the warm PDO phase for Tokelau (zero-time lag), American Samoa (one-year time lag), Kiribati (two-year time lag), Fiji and Vanuatu (one and two-year time lag), a negative relationship between ciguatera occurrence and the warm phase of the PDO was observed for New Caledonia at all time lags. By contrast, in Rarotonga, southern Cook Islands, the cool phase of the PDO between 1998 and 2008 was associated with increased occurrence of ciguatera from 1994 to 2010 with a two-year lag (Rongo and van Woesik, 2011). Similar to situation in the south Pacific islands, the present study showed a strong relationship between frequency of ciguatera cases and the warm phase of the PDO with a two-year lag time. The PDO alone with zero-, one-year- and two-year lag times were the best models, while no effect observed for models with SOI or El Niño alone. This differed to other studies that indicated a significant link between ciguatera cases and the SOI and El Niño (Llewellyn, 2010; Rongo and van Woesik, 2011). Ciguatera reported in Rarotonga showed a significant relationship to the SOI from 1992 to 2008, at one- and two-year lag times, and cases increased during El Niño periods in the warm PDO phase (Rongo and van Woesik, 2011). Llewellyn

(2010) also showed that the relationship between frequency of ciguatera and El Niño was similar to the PDO, while the SOI showed reversed relationships compared to the PDO for New Caledonia and Vanuatu. The warm phase of the PDO and frequency of ciguatera were positively related for the Pacific islands between latitudes 3 and 18 °S, while New Caledonia at 21 °S a decline in frequency of ciguatera was reported for the warm phase of the PDO. Similarly, ciguatera frequency was higher for the Pacific Islands between 3 – 15 °S during El Niño periods, but negatively related to ciguatera for French Polynesia and New Caledonia at 18 – 21 °S. The opposite relationship was reported for the effect of SOI (Llewellyn, 2010). Differences between the relationship of ciguatera frequency and climatic systems in the Pacific Islands may relate to regional differences in water circulation in the south Pacific, with circulation varying between El Niño and La Niña periods (Hales et al., 1999; Llewellyn, 2010; Rongo et al., 2009). The SSTs in Qld coastal waters have a latitudinal gradient (Heimann et al., 2011). It is likely that the SOI and El Niño had little effect in this study relative to the PDO, due to the regional difference in SSTs, as the Qld coastline extends from ~11 – 28 °S.

3.5.2 Effect of the PDO on coral bleaching history and its subsequent effect on frequency of ciguatera

Coral bleaching is triggered at levels of temperature and salinity similar to those that trigger growth of the ciguatera-causing benthic dinoflagellate, *Gambierdiscus* (Kibler et al., 2012; Sparrow et al., 2017). Increases in *Gambierdiscus* populations (Bagnis and Rougerie, 1992; Turquet et al., 2001) and incidence of ciguatera have, however, have been recorded 1-2 years after coral bleaching (Rongo and van Woesik, 2013). Whether ciguatera occurrence

is related to coral bleaching, independently of other environmental disturbances (cyclones, *A. planci* outbreaks), however, has received little attention. This study investigated the effect of coral bleaching on ciguatera occurrence in Qld, using the best climatic model accounting for the effect of the PDO at a two-year lag time. This model showed strong support for a decline in reported ciguatera cases years of coral bleaching. Death of coral provides additional substrate for settlement of algae and consequent recovery of *Gambierdiscus* populations on their preferred substrate, the algae. It is likely that *Gambierdiscus* populations would decline during periods of coral bleaching, as the thermal stress thresholds of the dinoflagellate and corals are similar. Recovery of *Gambierdiscus* populations have been documented between four and 17 months following warmer SSTs (Chateau-Degat et al., 2005; Turquet et al., 2001). The decline of *Gambierdiscus* during bleaching years was also observed at Rarotonga, Cook Islands (Rongo and van Woesik, 2013). While recovery of *Gambierdiscus* following periods of low salinity appears to depend on co-occurring benthic dinoflagellates (Sparrow et al., 2017), further research is needed to understand ecological and physiological responses to low salinity-induced coral bleaching events. On the GBR, low salinity-induced coral bleaching severely affected inshore reefs in the southern region, causing almost 100% mortality in 2011. Whether benthic dinoflagellates recover after periods of low salinity could be determined with reef-specific research.

3.5.3 Regional differences in major environmental disturbances

The frequency of disturbances and the severity of cyclones is predicted to increase under climate change conditions (Cheal et al., 2017; Hoegh-Guldberg, 1999; Hughes et al., 2003). Recently, environmental disturbances have been

shown to impact the diversity and abundance of fish assemblages, which declined with reduced structural complexity of coral reef habitats (Charbonnel et al., 2002; Cheal et al., 2017; Pratchett et al., 2011; Russ et al., 2015; Russ and Leahy, 2017). Cyclones can cause severe structural and biological disturbance to coral reefs, which may result in major loss of habitat, while coral bleaching and *A. planci* outbreaks have minimal impact on habitat complexity in the short-term, and therefore have less impact on available shelter and food resources for fish assemblages (Charbonnel et al., 2002; Cheal et al., 2017). Disturbances associated with coral bleaching, cyclones and *A. planci* outbreaks vary with latitude in frequency and intensity on the GBR, resulting in regional variations in the impact from different environmental disturbances (De'ath et al., 2012). At a regional-scale in Qld, the present study indicated a potential increase in frequency of ciguatera, 1-2 years after a severe cyclone, with ciguatera cases in the central and southern Qld regions most frequently impacted by coral bleaching and severe cyclones.

The loss of structural complexity of reef habitats in the central and southern GBR regions due to cyclones also resulted in large losses of mesopredators and smaller fish species, although abundances of grazing fish increased (Cheal et al., 2017). In Pacific island communities, increases in ciguatera cases were observed following increased abundance of grazing fish (Chinain et al., 2010; Rongo and van Woesik, 2013). Such fish are commonly consumed by Pacific Islanders and can increase in abundance following environmental disturbances to coral reefs. However, mesopredatory fish remain the major source of ciguatera cases in the tropics (Baumann et al., 2010; Morris et al., 1982; Stinn

et al., 2000) and the only group of fish implicated in Qld (Gillespie et al., 1986; Harvey, 1997). As disturbances, particularly cyclones, impact coral reef health and fish assemblages it is likely that disturbances may also disrupt the transfer of ciguatoxins through marine food webs. To determine the effect of environmental disturbances on the occurrence of ciguatera in Qld, regional questionnaires are needed in conjunction with regional and reef-specific research on critical ciguatera stages in marine food webs.

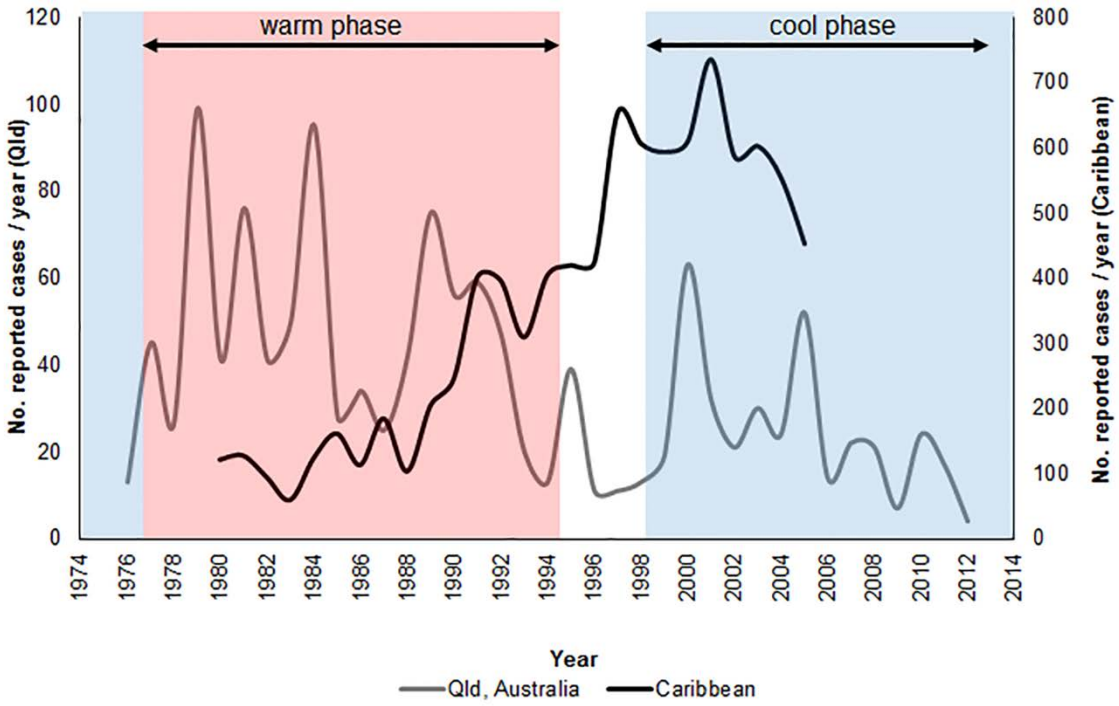

Figure 3.4 The frequency of reported ciguatera cases in Queensland (Qld), Australia (1976 – 2012, see Figure 3.1) and in the Caribbean between 1980 and 2005 (Caribbean Epidemiology Centre, 2008). The two oscillations of the PDO are shown by a blue shaded area for the cool phase (1974-1976; 1999-2003); a red area (1977 – 1995) to indicate a warm phase is unshaded; and the transition period between the two PDO phases is unshaded.

The frequency of ciguatera cases is driven by available benthic surfaces to host *Gambierdiscus* populations, abundance and diversity of grazing fish and invertebrates, as well as cultural and regional fish dietary choices. Ocean-scale climatic variations and environmental disturbances can affect the drivers of

ciguatera, and result in yearly fluctuations of reported cases, while available fish and invertebrate prey can influence the likely transfer of ciguatoxins through marine food webs. Abundance shifts in marine schooling fish species have been linked to the oscillation of the PDO phases, with higher sardine abundances in the warm phase and higher anchovy abundances in the cool phase (Chavez et al., 2003). In Qld, ciguatera cases are frequently associated with mesopredators, particularly Spanish mackerel and coral trout (Gillespie et al., 1986), for which Clupeidae are a major dietary component (Begg and Hopper, 1997; Beukers-Stewart and Jones, 2004; St John et al., 2001). There is, however, a scarcity of information on the distribution and abundance of schooling fish species on the GBR, and further research is needed to determine their importance in the trophic transfer of ciguatoxins. By contrast, in the Caribbean, the reverse was observed with higher frequency of ciguatera incidence during a cool, anchovy phase of the PDO (Figure 3.4). Barracuda have been frequently associated with ciguatera cases in Florida, USA and Puerto Rico (Escalona de Motta et al., 1986; Lawerence et al., 1980), and are known to predate anchovies (Schultze, 1983). It is, therefore, hypothesised that the trophic structure in marine food webs may play an integral role in the occurrence of ciguatera. To understand the influence of the PDO and other climatic systems on the frequency of reported ciguatera cases in the Pacific and the Caribbean, further research on abundance of schooling fish species, their dietary importance and relevance in the trophic transfer of ciguatoxins is essential. Multi-decadal research is important to identify critical stages in ciguatoxic marine food webs, the impact of climatic systems and environmental disturbances, and model sustainable solutions to support fisheries.

Chapter Four: Seasonal abundance of benthic toxic dinoflagellates in the central Great Barrier Reef[3]

4.1 Abstract

Ciguatera is a tropical illness that has expanded its geographic range through international travel, frozen tropical fish exports and the expansion of ciguatera-causing dinoflagellates into temperate coastal marine habitats. The drivers in the distribution of *Gambierdiscus* spp. and co-occurring dinoflagellates on their macroalgal substrates were investigated in this study. While macroalgal palatability and dinoflagellate associations were inconclusive, macroalgal thalli structure was identified as an important driver in the heterogenic distribution of *Gambierdiscus* populations. Higher *Gambierdiscus* densities were not always associated with fish-palatable macroalgal substrates. At mid-reef sites, *Gambierdiscus* populations were only found on flattened macroalgal substrates that were palatable to gastropods. Although there was no trend in substrate association, site-specific conditions appeared to play a role. It seems likely that grazing pressure and feeding technique play potential roles in the transfer of ciguatoxins within marine food webs. This needs further investigation to identify environmental and ecological factors that influence the composition and distribution of macroalgae and thereby the potential expansion of ciguatera-causing dinoflagellates into new coastal marine habitats. This research is

3 This chapter is adapted from:
Sparrow, L. and Heimann, K. (2016) Key environmental factors in the management of ciguatera. *Journal of Coastal Research*, 75:1007-1011.

The abstract, methods and results are presented as submitted, with the remainder of the chapter expanded to reflect the current state of knowledge in the field and modified to fit the thesis flow.

essential in developing ciguatera monitoring tools as marine fisheries are an integral part of the economy, diet and lifestyle of coastal populations throughout the world.

4.2 Introduction

Ciguatera is an illness induced when people consume contaminated reef fish. Marine fish species in tropical and subtropical regions bioconvert and trophically accumulate ciguatoxins derived from dinoflagellates of the genus *Gambierdiscus*. Between 50,000 and 500,000 people are affected annually worldwide (Arena et al., 2004; Chinain et al., 2010). Ciguatera incidence rates for tropical island nations have been based on reported ciguatera cases and/or questionnaires (Azziz-Baumgartner et al., 2012; Chateau-Degat et al., 2007; Lawerence et al., 1980; Skinner et al., 2011). When rates exceed 1,000 cases / 100,000 in a local population, the locality is referred to as a ciguatera hotspot. The islands of French Polynesia have consistently recorded such high rates, despite temporal fluctuations (Chinain et al., 2010; Skinner et al., 2011).

Variability in estimated ciguatera incidence rates is a function of factors in the probability of consuming contaminated fish. Variations in incidence rates can be triggered by factors such as seasonal migrations of predatory fish e.g. mackerel and barracuda. However other factors such as government bans and restrictions are likely to decrease incidence and the interaction of such factors can result in relatively stable rates (Figure 3.1). Affordable tourism-associated travel and increased demands for tropical fish exports are factors that affect incidence rates but also contribute to geographic expansion of ciguatera

(Heimann and Sparrow, 2015). Disturbance events, both natural and anthropogenic are influential factors (Figure 4.1) that can trigger temporary and permanent expansions of ciguatera-causing dinoflagellates, including *Gambierdiscus* spp.

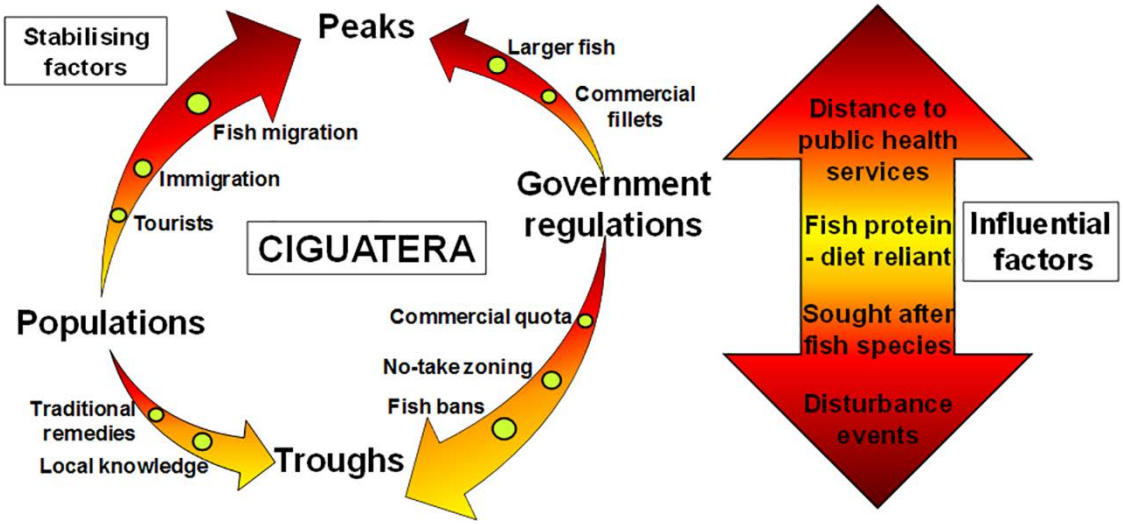

Figure 4.1 Schematic diagram of factors which interact to maintain roughly stable ciguatera incidence rates (left). Larger fluctuations in incidence rates are a response to influential factors (double arrow on right) that impact on the stable state.

Gambierdiscus spp. are found on coral reefs, frequently associated with macroalgae as a substrate and co-occur with other benthic dinoflagellates including *Prorocentrum* spp., *Ostreopsis* spp., and *Coolia* spp. Coral reefs are dynamic ecosystems where large-scale disturbance events such as cyclones, crown-of-thorns (*Acanthaster planci*) outbreaks and coral bleaching can lead to temporary or permanent phase shifts from coral- to macroalgal-dominated reefs (Heimann et al., 2011), which is thought to be followed by the range expansion of benthic dinoflagellates.

The colonisation of dead coral surfaces by algal turfs was recorded after coral bleaching events in Mayotte Island, Indian Ocean and in French Polynesia. A

lag of two to four months occurs before algal turfs were colonised by *Gambierdiscus* populations as accompanied by substantial increases in abundances (Bagnis and Rougerie, 1992; Turquet et al., 2001). By contrast, *Gambierdiscus* populations surveyed three months after a cyclone impacted Sudbury Reef, GBR, were only one-tenth the size of populations that were recorded three years prior to the cyclone (Lewis et al., 1986); unfortunately no information on macroalgal substratum abundance and diversity was provided. This variability in *Gambierdiscus* population responses to disturbance events may be related to the impact of the disturbance on the habitat complexity, as has been shown for reef-associated fish (Pratchett et al., 2011). Habitat destruction may displace existing *Gambierdiscus* populations along with their macroalgal substrata, thereby requiring more time for populations to re-establish prior to opportunistic increases associated with new surfaces. On the other hand, coral bleaching preserves structural habitat complexity and provides new surfaces for algae to colonise. Consequently, *Gambierdiscus* can readily transfer to new macroalgal surfaces.

The distribution and abundance of *Gambierdiscus* populations is thought to relate to preferences for their macroalgal substrata. Substratum preference studies have been based on taxonomic diversity and defence mechanisms to deter grazing by roving herbivorous fish in field surveys (Ballantine et al., 1985; Bomber et al., 1988a; Bomber et al., 1988b; Faust, 1995) and laboratory experiments (Nakahara et al., 1996; Parsons et al., 2011). Heterogenic distribution of *Gambierdiscus* populations have often been documented between and among macroalgal genera as well as within and between reefs

(Ballantine et al., 1985; Taylor, 1985). This patchy distribution on macroalgal substrates within reefs may simply be an artefact from recent grazing behaviours of roving herbivorous fish, which may inadvertently create localised disturbance of macroalgae, resulting in the redistribution of benthic dinoflagellates to the next closest macroalgal substrate.

Roving herbivorous fish differ in the range of macroalgae they find palatable. While generalist grazers, such as Siganidae (rabbitfish) find a diverse range of macroalgal species palatable, others, such as Scaridae (parrotfish), are more selective (Mantyka and Bellwood, 2007; Rasher et al., 2013). Grazing selectivity studies have shown that palatability of macroalgae may also vary within fish families. Scaridae generally have a high preference for the green alga *Halimeda* spp. *Scarus schlegeli* and *Chlorurus sordidus* engage in low to medium levels of grazing on brown algae, *Dictyota, Padina, Sargassum* and *Turbinaria*, taxa which were avoided by other scarids in the same genera (Mantyka and Bellwood, 2007; Rasher et al., 2013). Chinain et al. (2010) noted that several fish associated with reported ciguatera cases in French Polynesia, including the scarids *Scarus* spp. and *Chlorurus microrhinos*, avoided grazing on calcareous red algae. However, highest densities of *Gambierdiscus* were found on the calcareous red alga, *Jania* sp. (Chinain et al., 2010). This suggests that palatability may play a role in the uptake of *Gambierdiscus* spp. Scaridae have been frequently associated with ciguatera incidence in the Pacific (Clua et al., 2011), however, there is limited information on grazing pressure relative to the distribution of *Gambierdiscus* populations on macroalgal substrates. While herbivorous fish are thought to be the major vectors in the transfer of

ciguatoxins into marine food webs, the importance of invertebrate herbivores, also potential grazers of benthic dinoflagellates, remains under-investigated (Cruz-Rivera and Villareal, 2006; Lewis, 2006). Cruz-Rivera and Villareal (2006) reviewed *Gambierdiscus toxicus* abundance on a range of macroalgae and tested their palatability among grazers, including fish and meso- and micro-invertebrate grazers. The picture becomes even more complex given that light intensities and water dynamics also appear to impact on macroalgae-*Gambierdiscus* associations.

The optimal growth rates for several *Gambierdiscus* spp. are at irradiances ≥ 231 µmol photons m^{-1}. s^{-1} (Kibler et al., 2012; Villareal and Morton, 2002). Villareal and Morton (2002) proposed that thallus structure of macroalgal substrata may provide optimal light intensities through shading of *Gambierdiscus toxicus*. This research hypothesised that the type of reef, such as fringing reefs, cays, inshore and outer reef systems; as well as the position on a reef, such as reef flat, crest or lagoon, obviously also relates to light intensities received and water dynamics. It appears that in sheltered positions, such as lagoons and reef flats, benthic dinoflagellates, including *Gambierdiscus* spp. may associate with flattened or terete thalli more than other substrates. By contrast, in high energy locations, such as reef crests and windward side of reefs, leathery thalli may be the only available substrate.

On the Great Barrier Reef (GBR), cyclones can result in extensive flood plumes with nutrient-rich waters triggering trophic blooms. Coral bleaching events are a consequence of warmer sea surface temperatures that can also lead to

population growth of *Gambierdiscus* spp. and co-occurring benthic dinoflagellates (Heimann et al., 2011). Positive correlations have been shown between warmer sea surface temperatures and the increase in ciguatera incidence (Hales et al., 1999; Llewellyn, 2010; Tester et al., 2010), as have the frequency and intensity of large-scale disturbance events (Rongo and van Woesik, 2011).

Climate change affects weather patterns, extending warmer sea surface temperatures and strengthening ocean currents into temperate waters, which promote temporary and permanent range expansion of ciguatera-causing dinoflagellates into new coastal habitats (Heimann et al., 2011). However, reasons for the patchy distribution of *Gambierdiscus* spp. and co-occurring benthic dinoflagellates on macroalgal substrates are still poorly understood.

This study hypothesised that the patchy distribution is likely driven by predation as well as thallus structure of macroalgal substrata. The aims of this study were to investigate potential macroalgal substratum associations in the distribution of benthic dinoflagellates.

4.3 Methods

Two separate surveys were conducted in the central GBR during 2008 – 2009. One survey was conducted in Nelly Bay, Magnetic Island (Figure 4.2), with samples collected monthly as weather conditions permitted. Nelly Bay is on the south-eastern side of Magnetic Island and has a sub-tidal fringing reef.

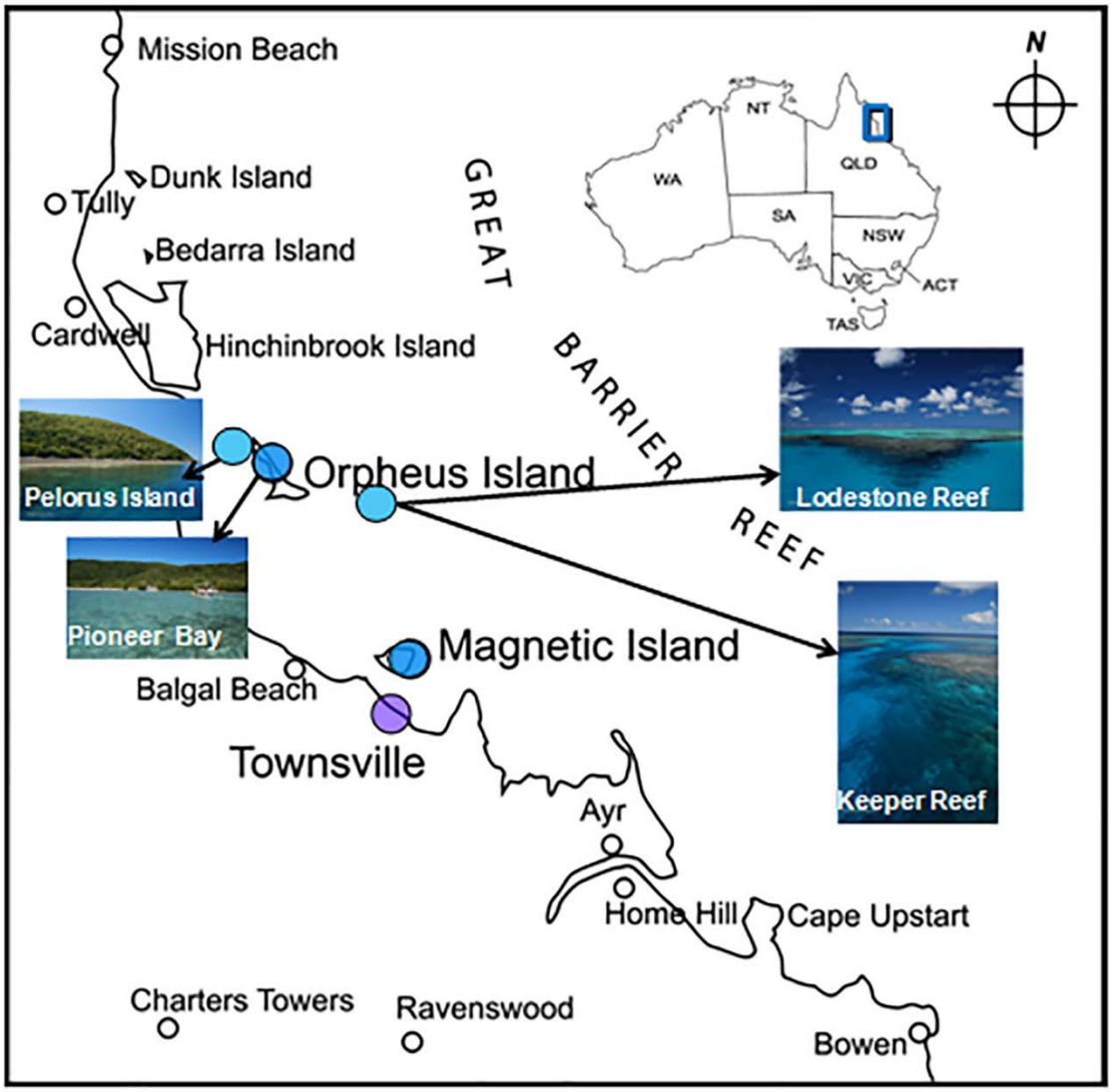

Figure 4.2 The location of survey sites on the fringing reef at Nelly Bay, Magnetic Island and on Orpheus and Pelorus Islands, as well as two mid-reef sites, in the central Great Barrier Reef, Australia.

The second survey was based at Orpheus Island Research Station (OIRS) and was conducted on three separate 10-day periods in November 2008, March and August 2009, to represent seasonal differences between the Austral wet and dry seasons (Heimann et al., 2010). Data from four of the sites collected in August 2009, as part of the Austral dry season, are reported here: two mid-shelf reefs: Lodestone and Keeper Reefs; and two inshore-reefs: Pioneer Bay on Orpheus Island and Pelorus Island (Figure 4.2). The fringing reef at Pioneer Bay had an extensive inter-tidal reef flat, while the fringing reef at Pelorus Island

was sub-tidal, remaining submerged at low-tide. Benthic toxic dinoflagellates were collected from their macroalgal substrates, which were sampled and processed as described by Heimann et al. (2010). All benthic dinoflagellate cell counts were standardised as mean abundance per wet weight (g) of macroalgal substratum sampled and to number of macroalgal samples collected.

To determine if benthic dinoflagellates had an association between macroalgal substrata, macroalgae were firstly investigated by thalli structure, with three categories: flattened; leathery; or terete. Secondly, by palatability of macroalgae between grazers: fish; urchins; crustaceans; gastropods; mesograzers; and sea turtles (Cruz-Rivera and Villareal, 2006).

4.4 Results

Total abundance of benthic dinoflagellates at Nelly Bay, Magnetic Island, was variable over the months surveyed with the highest abundance in May 2008 (1914 cells/g wet weight (ww)-substrate) followed by a decrease in June to 96 cells/g. ww-substrate (Figure 4.3A). Higher benthic dinoflagellates abundance at 1406 cells/g ww-substrate also occurred in October 2009 (Figure 4.3A).

Composition of the benthic dinoflagellates community was comparable within all months sampled at Nelly Bay and was dominated by the dinoflagellate, *Ostreopsis* spp. (Figure 4.3A). Although *Gambierdiscus* spp. were a minor component of the benthic dinoflagellates community, an increase in abundance was observed in August 2008.

The total abundance of benthic dinoflagellates at Pioneer Bay for August 2009 (Orpheus Island Research Station (OIRS) based survey) was comparable to the total abundance for Nelly Bay in August 2008 (Figure 4.3A and D). Total benthic dinoflagellates abundances were significantly different between the mid-reefs, Lodestone and Keeper Reefs ($T\text{-test}_{df28}$; $p<0.05$); as well as between inshore-reefs ($T\text{-test}_{df28}$; $p<0.05$) at Pioneer Bay and Pelorus Island (Figure 4.3D). Similar to Nelly Bay, *Gambierdiscus* spp. were a minor component of the benthic dinoflagellates community; however, by contrast, *Prorocentrum* spp. dominated (Figure 4.3A and D).

There was no clear seasonal trend in substratum association for benthic dinoflagellates when based on macroalgae thalli structure for Nelly Bay (Figure 4.3B). Flattened macroalgal substrates were more frequently associated with higher benthic dinoflagellates abundances at Lodestone Reef and Pioneer Bay for August 2009 (Figure 4.3E). Benthic dinoflagellates abundances were higher on terete macroalgal substrates at Keeper Reef and Pelorus Island, which was similar to benthic dinoflagellates associations observed at Nelly Bay in August 2008 (Figure 4.3B and E).

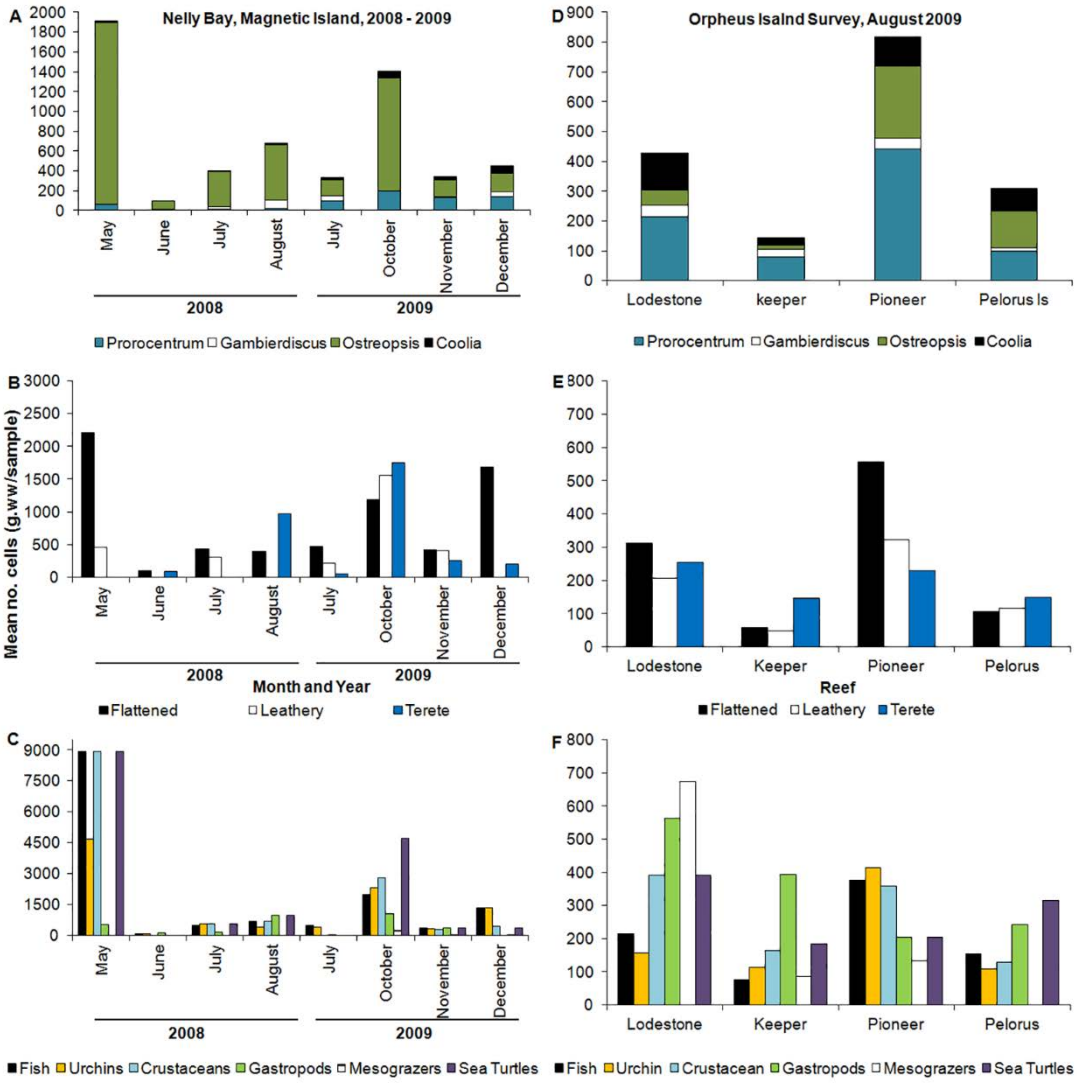

Figure 4.3 Mean abundances of benthic toxic dinoflagellates observed in two surveys: monthly seasonal survey at Nelly Bay, Magnetic Island, 2008 - 2009 (A-C); and the Orpheus Island Research Station-based survey, August 2009 (D-F). For both surveys, mean benthic toxic dinoflagellate abundances are shown: for community composition (A and D); with preference for macroalgal growth morphologies (B and E); and for known palatability of grazers on surveyed macroalgal substrata (C and F). Mean abundances were standardised to macroalgal substrata by wet weight (g ww) and number of samples.

Benthic dinoflagellates abundances were often associated with macroalgae known to be palatable to crustaceans, urchins and sea turtles as well as fish in all months surveyed at Nelly Bay (Figure 4.3C). In comparison, macroalgal substrata palatable to fish and urchins had the least benthic dinoflagellates abundances associated at both Lodestone and Keeper Reefs (Figure 4.3F). Higher benthic dinoflagellates abundances were associated with macroalgal

substrata known to be palatable to gastropods at Lodestone and Keeper Reefs as well as Pelorus Island. Macroalgal substrata known to be palatable to mesograzers recorded highest benthic dinoflagellates abundances at Lodestone Reef but were low or absent at other reefs (Figure 4.3F). At Pioneer Bay, higher benthic dinoflagellates abundances occurred on macroalgal substrates known to be grazed by urchins as well as fish and crustaceans (Figure 4.3F).

Table 4.1 Mean abundances of *Gambierdiscus* cells (number of cells/g ww of sample) relative to macroalgal growth morphologies and known grazer palatability (Cruz-Rivera and Villareal, 2006) at Nelly Bay, Magnetic Island in 2008 and 2009.

	2008				2009			
	May	**June**	**July**	**August**	**July**	**October**	**November**	**December**
Fish								
Flattened			17.67	23.96	118.49	4.44	6.88	197.60
Leathery			11.72				6.50	
Terete				135.14			26.09	37.35
Urchins								
Flattened			17.67	23.96	118.49	5.92	6.88	197.60
Leathery	2.88							
Terete							4.22	37.35
Crustaceans								
Flattened			17.67	23.96		4.44	2.30	42.74
Leathery								
Terete				135.14			26.09	37.35
Gastropods								
Flattened			68.34			1.72		
Leathery								
Terete				135.14			27.75	
Mesograzers								
Flattened								
Leathery								
Terete						1.81	6.61	3.41
Sea Turtles								
Flattened			17.67					
Leathery								
Terete				135.14			26.09	37.35

Gambierdiscus cells were absent in June 2008 at Nelly Bay (Table 4.1) and the lowest abundances were recorded in May 2008, however showing the highest benthic dinoflagellates abundance (Figure 4.3B and Table 4.1). In May 2008, *Gambierdiscus* cells were only recorded on leathery macroalgae that were

palatable to urchins (Table 4.1). By contrast, benthic dinoflagellates cells were more frequently associated with flattened macroalgae known to be palatable to fish, crustaceans and sea turtles (Figure 4.3B and C) (Cruz-Rivera and Villareal, 2006).

Higher abundances of *Gambierdiscus* cells were associated with flattened macroalgal substrata known to be grazed by gastropods (Cruz-Rivera and Villareal, 2006) in July 2008 and by fish and urchins in July and December 2009 (Table 4.1). In August 2008 and November 2009, higher abundance of *Gambierdiscus* cells were associated with terete macroalgal substrates known to be grazed by fish, crustaceans, gastropods and sea turtles (Cruz-Rivera and Villareal, 2006). This was similar to frequent high benthic dinoflagellates abundances with terete substrates in August 2008, but contrasted with November 2009 when terete was least colonised by benthic dinoflagellates (Figure 4.3B and Table 4.1).

Highest abundance of *Gambierdiscus* cells within the OIRS-based survey was recorded at Lodestone Reef and was associated with terete macroalgal substrata known to be palatable to crustaceans and sea turtles (Table 4.2) (Cruz-Rivera and Villareal, 2006). Higher *Gambierdiscus* spp. abundances were associated with terete macroalgal substrates compared with highest benthic dinoflagellates abundances associated with flattened macroalgal substrata at Lodestone Reef and Pioneer Bay (Figure 4.3E and Table 4.2). Macroalgal substrata known to be palatable to urchins (Cruz-Rivera and Villareal, 2006) was least colonised by *Gambierdiscus* cells at Lodestone and Keeper Reefs but

were colonised in association with terete and leathery macroalgal substrata at Pioneer Bay (Table 4.2). High benthic dinoflagellates and *Gambierdiscus* abundances were recorded for macroalgal substrata known to be palatable to gastropods (Cruz-Rivera and Villareal, 2006) at both mid-reefs. However, *Gambierdiscus* cells were recorded on flattened macroalgae only known to be gastropod palatable (Table 4.2) (Cruz-Rivera and Villareal, 2006).

Table 4.2 Mean abundances of *Gambierdiscus* cells (number of cells/g ww of sample) relative to macroalgal growth morphologies and known grazer palatability (Cruz-Rivera and Villareal, 2006) at mid- and inshore-reefs during the OIRS based survey.

	Mid-reefs		Inshore-reefs	
	Lodestone Reef	Keeper Reef	Pioneer Bay	Pelorus Island
Fish				
Flattened			3.12	11.16
Leathery	8.93	1.59	10.13	
Terete	35.04	26.33	16.72	138
Urchins				
Flattened			3.12	9.33
Leathery	8.93	1.59	18.69	
Terete	4.01	13.87	20.29	2.31
Crustaceans				
Flattened				5.39
Leathery				
Terete	50.56	46.49	6.04	
Gastropods				
Flattened	29.38	23.38		
Leathery				
Terete	20.22	34.93	6.04	
Mesograzers				
Flattened				
Leathery	8.93	1.59		
Terete	26.52			
Sea Turtles				
Flattened				8.09
Leathery				
Terete	50.56	63.84	6.04	

4.5 Discussion

It is generally hypothesised that herbivorous fish grazing on macroalgae inadvertently transfer *Gambierdiscus* spp. and co-occurring benthic dinoflagellates into marine fish food webs, with their toxins then being bio-

converted into ciguatoxins and bioaccumulated into larger predatory fish (Heimann et al., 2011). However, links in the transfer of ciguatoxins from *Gambierdiscus* populations into marine fish food webs in the GBR remain unclear. This study identified potential key parameters in the distribution of *Gambierdiscus* populations in the central GBR.

The hypothesis that fish grazing on macroalgae transfer ciguatoxins into fish food webs implies that *Gambierdiscus* populations are uniformly distributed on macroalgal substrata. In this study, benthic dinoflagellate populations, and in particular *Gambierdiscus* spp, had a heterogenic distribution on macroalgal substrates. Previous field surveys have shown similar heterogeneity in *Gambierdiscus* spp. distribution on macroalgal substrata (Ballantine et al., 1985; Chinain et al., 1999b; Gillespie et al., 1985). Distribution patterns for *Gambierdiscus* spp. or benthic dinoflagellate assemblages based on macroalgal palatability were inconclusive in this study. Instead, distribution patterns suggested site-specific conditions may play a role. This may be due to a number of factors relative to abundance and diversity of herbivores at a site. Diversity of herbivorous fish rather than abundance was shown to minimise macroalgal growth and maintain coral dominance on reefs (Rasher et al., 2013). A higher diversity of herbivorous fish at a reef is likely to be attracted by a broader range of macroalgal species palatable to them. Thereby, grazing may potentially lower diversity of macroalgae as available substrates for benthic dinoflagellates, however, this is also dependent on whether the herbivorous fish are general or selective grazers. High abundance of generalist grazers such as Siganidae (rabbitfish) may reduce the diversity of macroalgal substrata

available for benthic dinoflagellates. In comparison, high abundance of specialised or highly selective herbivores, such as Scaridae (parrotfish), could result in an increased diversity of macroalgal substrata as grazing is targeted to a limited range of macroalgal species. Therefore, grazing pressure and macroalgal palatability combined could hypothetically dictate availability of macroalgal substrates available for *Gambierdiscus* spp. and co-occurring benthic dinoflagellates (Figure 4.4).

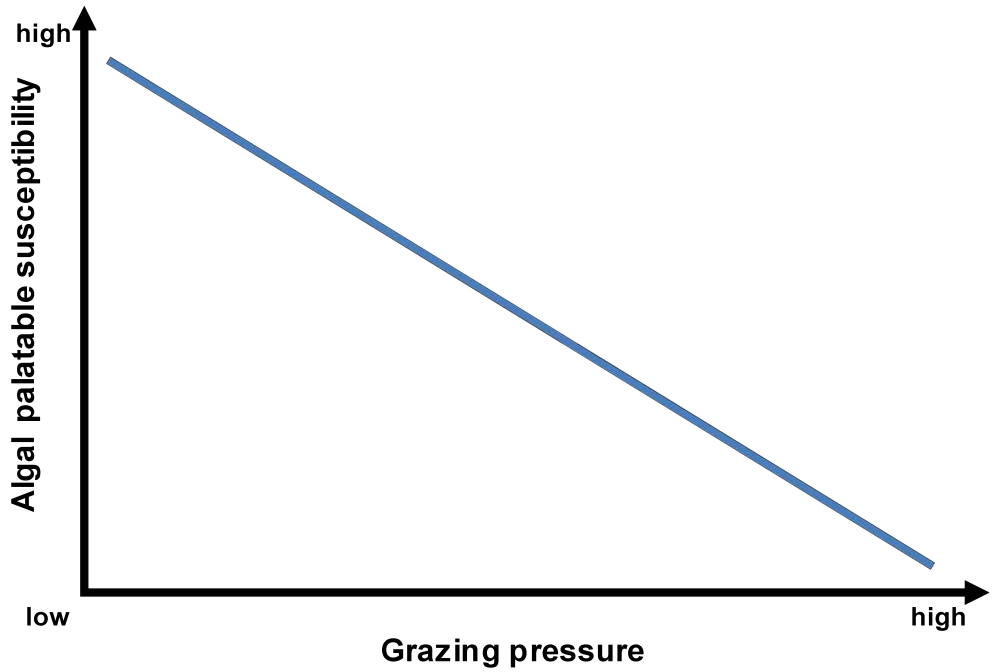

Figure 4.4 Schematic diagram of macroalgal substrate abundance and diversity for benthic dinoflagellates based on palatability of macroalgae against grazing pressures by herbivorous fish.

Grazing selectivity by herbivores may vary depending on availability of their preferred macroalgae. It was noted that when *A. planci* were in low abundance, they were selective coral predators and prefer to feed on *Acropora* spp. (De'ath and Moran, 1998). However, when an *A. planci* outbreak occurred on a reef, the high abundance necessitates that the sea star be less selective, feeding on numerous coral genera (De'ath and Moran, 1998; Kayal et al., 2012). Similarly,

it is argued that highly selective herbivores are likely to change to a more generalised diet in a scenario of high abundance combined with high diversity of herbivores. This scenario is likely to increase grazing pressure at a reef, necessitating the grazing of less palatable or even previously avoided macroalgal species by both general and the previously highly selective herbivores. In such scenarios, the limited availability of macroalgal substrata for benthic dinoflagellates may result in the association with other benthic surfaces, such as sediments and coral rubble. In this study, macroalgal substrata palatable to fish, urchin and crustaceans were frequently dominant at Nelly Bay, Magnetic Island, which is characterised by inshore stands of *Sargassum* spp. (Sparrow pers. obs.). Herbivory exclusion experiments showed macroalgal composition changed in response to grazing pressures (Diaz-Pulido and McCook, 2002a; McClanahan et al., 2002). Exclusion of larger herbivorous fish conducted at Orpheus Island resulted in an increase in abundance and dominance of leathery macroalgae of the genus, *Sargassum*. which were grazed immediately once the exclusions were removed (Hughes et al., 2007). Similarly, *Sargassum* dominance at Nelly Bay may suggest low grazing pressure or absence of larger herbivorous fish (Figure 4.4). Abundance of herbivorous fish are known to be higher at mid-reef sites compared to inshore reefs (Russ, 1984). In this study, macroalgae palatable to fish and sea urchins were located inshore at Nelly Bay and Pioneer Bay, while gastropod, mesograzer and sea turtle palatable macroalgal substrates were located at mid-reefs. Within the time of this study, Loeffler et al. (2015) demonstrated that grazing by herbivorous fish negatively affected *Gambierdiscus* populations but the study has limited applicability due to the broad categories chosen for fish,

such as herbivore, piscivores, invertivore. Predation by grazers other than herbivorous fish is thought to contribute in the transfer of ciguatoxins into fish food webs (Cruz-Rivera and Villareal, 2006; Heimann et al., 2011), leading to the patchy distribution of benthic dinoflagellates on macroalgal substrates. The higher abundances of *Gambierdiscus* populations found on flattened macroalgal substrates at the mid-reefs, Lodestone and Keeper Reefs in this study were only palatable to gastropods. This may indicate high herbivorous fish but low gastropod abundance at mid-reefs (Klumpp and Pulfrich, 1989). In Nelly Bay, higher *Gambierdiscus* densities were found on macroalgal substrates palatable to crustaceans, urchins and gastropods, as well as fish and varied between months. This pattern of *Gambierdiscus* distribution and abundance may indicate non-selective grazing, leaving only remnants of macroalgal stands for sampling. Further field and laboratory experiments are needed to investigate the impact of fish and invertebrate grazing on the distribution of *Gambierdiscus* and co-occurring benthic dinoflagellates on macroalgae. This is essential in identifying key vectors in the transfer of ciguatoxins into fish food webs.

In laboratory experiments, *Gambierdiscus* spp. displayed different degrees of motility and attachment to a range of individual macroalgal substrates (Nakahara et al., 1996; Parsons et al., 2011; Rains and Parsons, 2015). The behaviour between motile and attached cells may be another factor that affects the heterogenic distribution of *Gambierdiscus* spp. It is proposed that grazers are more likely to disperse *Gambierdiscus* cells that are highly motile on palatable macroalgal substrates, while a higher degree of attachment to macroalgal substrates is likely to increase uptake of *Gambierdiscus* cells by

grazers. Herbivorous fish encompass a range of feeding techniques, including scrapers, excavators, detritivores, croppers and farmers (Charbonnel et al., 2002), which can vary not just between fish families, but also species (Tebbett et al., 2017). Standing *Gambierdiscus* population sizes may be influenced by grazing technique. Indeed, Loeffler et al. (2015) demonstrated that grazing pressure effects *Gambierdiscus* spp. populations, although piscivore and invertivore fish were also prominent. Acanthuridae (surgeonfish) and Scaridae form part of the diet in Rarotonga, Cook Islands and are frequently associated with ciguatera incidence (Rongo and van Woesik, 2013). Acanthurids were observed to increase in abundance following disturbances and observed to target filamentous turf algae (Rongo and van Woesik, 2013). Particularly, the acanthurid, *Ctenochaetus striatus*, a common detritivore on Rarotonga, is considered a high-risk ciguatera species; while *Acanthurus nigrofuscus* is a common cropper and considered one of several acanthurids to be low-risk. Both acanthurids preferentially target turf algae, however, only abundance of *C. striatus* was positively correlated with reported ciguatera cases (Rongo and van Woesik, 2013). Tebbett et al. (2017) demonstrated that acanthurid, *C. striatus* targeted the sediment matrix within the turf algae, while *A. nigrofuscus* only cropped the turf algal filaments. This suggests that benthic dinoflagellates, particularly *Gambierdiscus* spp., are likely to be found within the matrix rather than on the turf algal filaments above the matrix.

In Queensland (Qld), Australia, acanthurids, *C. striatus* and *A. nigrofuscus* as well as several parrotfish are commonly found on the GBR, with abundances and diversity increasing from inshore to outer reefs (Hoey and Bellwood, 2008;

Russ, 1984). These fish species have not been implicated in reported ciguatera cases in Qld because they do not form part of the western diet. In contrast to Rarotonga, Cook Islands, Serranidae, (including grouper and coral trout, *Plectropomus* spp.) and Scombridae (mackerel) are frequently associated with reported ciguatera cases in Qld, Australia (Gillespie et al., 1986; Harvey, 1997). Therefore, on the GBR, it is suggested that serranids and scombrids would predate acanthurids, particularly *C. striatus*, as potential ciguatoxin vectors. Dietary studies for serranids on the GBR (Beukers-Stewart and Jones, 2004; Kingsford, 1992; St John, 1999, 2001; St John et al., 2001) indicate that diversity of fish predated was greater in *Plectropomus leopardus* compared to *Cephalopholis* spp. (Figure 4.5A and B). While acanthurids were a minor component of the diet for *P. leopardus* (Figure 4.5A), these grazers were absent from the diets for *Cephalopholis* spp. (Figure 4.5B) Acanthurids were an important component of the diet for *C. argus* in the Red Sea, however, only *A. nigrofuscus* were targeted (Shpigel and Fishelson, 1989); which was considered low risk for ciguatera in Rarotonga. The diet of scombrid species on the GBR also appear to have an absence of acanthurids with Clupeidae (sardines, herrings and anchovies) mainly targeted (Begg and Hopper, 1997). To determine if *C. striatus* or other grazers are a key vector of ciguatoxin transfer in marine food webs on the GBR, further studies are needed that target the high-risk for ciguatera fish on the GBR, serranids and scombrids. In addition, it is suggested that understanding fish feeding techniques and associations is essential to identify key vectors in the transfer of ciguatoxins into marine food webs. Substrate associations for *Gambierdiscus* spp. are likely to remain

inconclusive without research on fish species, particularly region-specific high-risk for ciguatera poisoning, as observed by Rongo and van Woesik (2013).

Gambierdiscus population densities were also found to vary between macroalgal thalli structures, which differed between sites. Higher densities of benthic dinoflagellates were associated with flattened substrates at inshore-reefs, terete substrates at mid-reefs and varied between months in Nelly Bay, Magnetic Island. This study is the first to investigate substrate association by macroalgal thalli structure, which has flow-on effects in addition to herbivory pressures. Associations with thalli structure may be a response to regulation required for irradiance levels depending on reef characteristics. Flattened thalli structure enables shading from high irradiance for benthic dinoflagellates (Villareal and Morton, 2002), which may occur on shallow inter-tidal reef flats, such as in our study at Pioneer Bay, Orpheus Island and on mid-reefs that are shallow or partially exposed at low tides, observed at Lodestone Reef. In comparison, association with terete thalli structure at inter-tidal, submerged reefs on Pelorus Island and at Keeper Reef in our study may enable more variable regulation of irradiance associated with fluctuating levels of water depth during the day. Nelly Bay is an inter-tidal submerged inshore reef with stands of *Sargassum* spp. present near-shore. This may reduce the effects of turbulence for macroalgae with flattened thalli structure, such as *Dictyota* dichotoma, *Padina* spp. and *Lobophora* spp., which were observed within and beneath the *Sargassum* spp. stands. The variation in monthly association with different thalli structure at Nelly Bay may be promoted by low grazing pressure, which is associated with the proliferation of *Sargassum* spp. (Hughes et al., 2007). Low

grazing pressure may in turn enable substrate selectivity to be driven by environmental factors that influence growth of macroalgae, such as nutrients, temperature and freshwater inputs.

Large-scale disturbance events have been shown to positively correlate with increases in ciguatera incidence (Llewellyn, 2010; Rongo and van Woesik, 2011). The impact of small-scale disturbances on the distribution and abundances of benthic dinoflagellates, in particularly *Gambierdiscus* spp., such as fishing, wave action from boat traffic and competition for space on reefs are poorly understood. Competition for space between corals and macroalgae is well known (McCook, 2001), however, competition between macroalgal thalli structures have not been explored, such as growth rates, overshadowing and abrasive action. The influence of these factors on macroalgal composition is likely to have a flow-on effect to the distribution of benthic dinoflagellates.

Gambierdiscus spp. are frequently observed to co-occur on macroalgal substrates with other benthic dinoflagellates (Heimann et al., 2011), however, the role of species-specific interactions, such as allelopathy are poorly understood (see chapter five). This study showed that higher *Gambierdiscus* populations were not always associated with the same macroalgal thalli structure for other benthic dinoflagellate populations. In Pioneer Bay, higher *Gambierdiscus* abundances were on terete macroalgal substrates, which were least colonised by other benthic dinoflagellate populations. Highest benthic dinoflagellate abundance was on flattened macroalgal substrates in May 2008 at Nelly Bay, however, *Gambierdiscus* cells were only recorded on leathery

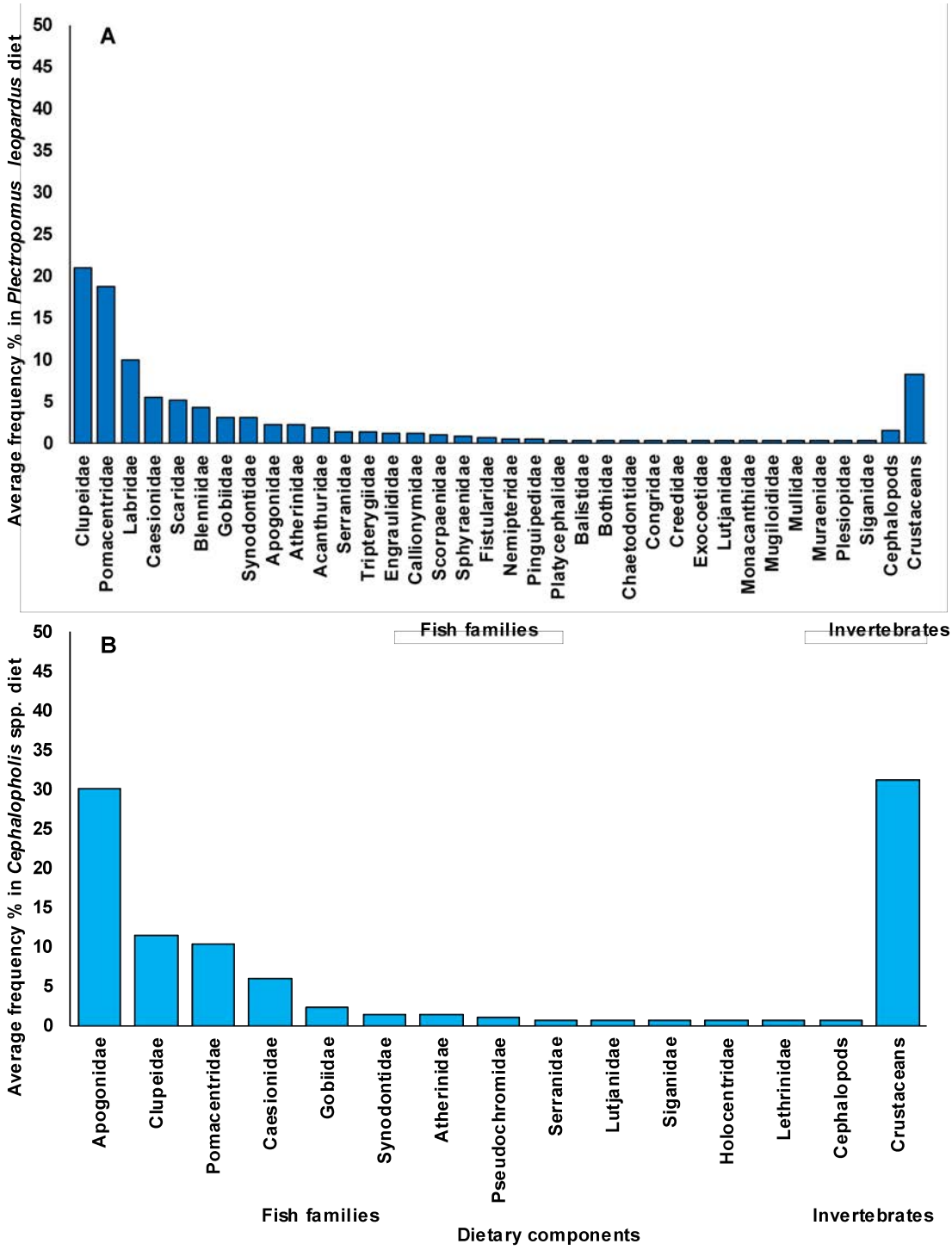

Figure 4.5 Average percentage frequency of dietary components for Serranidae, (A) *Plectropomus leopardus* and (B) *Cephalopholis* spp. (Beukers-Stewart and Jones, 2004; Kingsford, 1992; St John, 1999, 2001; St John et al., 2001).

macroalgal substrates there. Allelopathic interactions have been shown to influence composition of temperate bloom-forming toxic dinoflagellates (Hakanen et al., 2014). The dominance of *Ostreopsis* spp. in Nelly Bay and

Prorocentrum spp. at mid-shelf and inshore reefs during the OIRS-based survey conducted August 2009, were also observed by Skinner et al. (2013), with similar benthic dinoflagellate dominance patterns, although generally higher abundances were observed in this study. Lower abundances reported by Skinner et al. (2013) may potentially be an artefact of the limited range of macroalgal species sampled or an underestimation due to loss from using a larger mesh sieve (38 µm). Other studies have also noted that use of sieves with pore size of > 20 µm can result in underestimating abundance of benthic dinoflagellate genera (Bomber et al., 1989; Grzebyk et al., 1994). Production of mucilage by benthic dinoflagellates is thought to assist in attachment to substrates, as free-swimming *Gambierdiscus* spp. have sometimes been observed to remain attached by a mucilage thread (Nakahara et al., 1996). Toxins within the mucilage produced by *Gambierdiscus* sp. are thought to inhibit the growth of co-occurring diatoms (Bomber, 1990). Toxin-containing exuded mucilage may deter micro- or meso-grazers, such as crustaceans that co-occur with benthic dinoflagellates on macroalgal substrates. Alternatively, this may reduce grazing pressure on their host macroalgal substrate, similar to coral symbionts, which reduce predation from *A. planci* on its coral host (Pratchett, 2001).

In summary, the heterogenic distribution of *Gambierdiscus* spp. populations may be driven by several biological factors, including grazing pressures, feeding techniques of grazing fish, including detritivores and invertivores, as well as the potential role of co-occurring benthic dinoflagellates against predation for the macroalgal host as well as the dinoflagellates themselves. In this context,

disturbance events and climatic conditions can influence macroalgal succession and range expansion of macroalgae and *Gambierdiscus* populations (Boada et al., 2010; Hallegraeff, 2010; Kohli et al., 2014).

Range expansions of *Gambierdiscus* populations in tropical regions following large-scale disturbance events have been correlated with increases in ciguatera incidence (Chateau-Degat et al., 2005). The thallus structure and palatability of macroalgal substrates have an important role in the distribution and abundance of *Gambierdiscus* populations. To understand the significant role these factors play in ciguatoxin transfers in marine food webs, it is essential to explore the role of herbivory pressures, herbivorous fish and invertebrate diversity and abundances, competition and allelopathic interactions on macroalgal composition and abundance.

Changed weather patterns under current climate warming conditions have promoted the expansion of *Gambierdiscus* populations into temperate coastal habitats (Hallegraeff, 2010; Kibler et al., 2015). There is concern that colonisation by *Gambierdiscus* spp. into temperate coastal habitats will be followed by ciguatoxins accumulating in local fish populations and fisheries. It is therefore, essential to continue investigating key factors that influence distribution of *Gambierdiscus* populations and key vectors in the transfer of ciguatoxins into fish food webs. Without this, monitoring of reported ciguatera cases and implementing fisheries management strategies, such as bans and restrictions, will not prevent the likely impact from the continued geographic expansions by *Gambierdiscus* spp. and potential expansion of ciguatera.

Chapter Five: Temperature and salinity tolerance of two strains of *Gambierdiscus carpenteri*, isolated from the central Great Barrier Reef[4]

5.1 Abstract

Increases in reported incidence of ciguatera fish poisoning (hereafter ciguatera) have been linked to warmer sea temperatures that are known to trigger coral bleaching events. The drivers that trigger blooms of ciguatera-causing dinoflagellates on the Great Barrier Reef (GBR) are poorly understood. This study investigated the effects of increased temperatures and lowered salinities, often associated with environmental disturbance events, on the population growth of two strains of the potentially ciguatera-causing dinoflagellate, *Gambierdiscus carpenteri* (NQAIF116 and NQAIF380). Both strains were isolated from the central GBR with NQAIF116 being an inshore strain and NQAIF380 an isolate from a stable environment of a large coral reef aquarium exhibit in ReefHQ, Townsville, Australia. Species of *Gambierdiscus* are often found as part of a mixed assemblage of benthic toxic dinoflagellates on macroalgal substrates. The effect of assemblage structure of dinoflagellates on the growth of *Gambierdiscus* populations has, however, not been explored. The study, therefore investigated the growth of *G. carpenteri* within mixed assemblages of benthic dinoflagellates. Population growth was monitored over

4 This chapter is adapted from:
Sparrow, L., Momigliano, P., Russ, G. R., and Heimann, K. (2017) Effects of temperature, salinity and composition of the dinoflagellate assemblage on the growth of *Gambierdiscus carpenteri* isolated from the Great Barrier Reef. Harmful Algae 65:52-60.

The chapter has been modified to fit the thesis flow.

a period of 28 days under three salinities (16, 26 and 36) and three temperature (24, 28 and 34°C) conditions in a fully crossed experimental design. Temperature and salinity had a significant effect on population growth. Strain NQAIF380 exhibited significantly higher growth at 28°C compared to strain NQAIF116, which had highest growth at 24°C. When strain NQAIF116 was co-cultured with the benthic dinoflagellates, *Prorocentrum lima* and *Ostreopsis* sp., inhibitory effects on population growth were observed at a salinity of 36. In contrast, growth stimulation of *G. carpenteri* (strain NQAIF116) was observed at a salinity of 26 and particularly at 16 when co-cultured with *Ostreopsis*-dominated assemblages. Range expansion of ciguatera-causing dinoflagellates could lead to higher frequency of reported ciguatera illness in populated temperate Australian regions, outside the tropical range of the GBR. Therefore, our findings on salinity and temperature tolerance of two strains of *G. carpenteri* indicates potential adaptability to different local environmental conditions. These are baseline data for future investigations into the potential southward range expansion of ciguatera-causing dinoflagellates originating from the GBR.

5.2 Introduction

Ciguatera is a human illness induced by the consumption of fish that have accumulated ciguatoxins through their diet. Ciguatoxin analogues are produced by benthic toxic dinoflagellates of the genus *Gambierdiscus*, potentially being bio-converted into more potent ciguatoxins after consumption by fish (Heimann et al., 2011; Lewis et al., 1991; Tester et al., 2013). Species of *Gambierdiscus* frequently co-occur with other benthic dinoflagellates, including *Prorocentrum* and *Ostreopsis*, the latter two species often being dominant, and these

assemblages commonly utilise macroalgae as a substrate, but are also known to colonise other benthic surfaces including coral and sand (Faust, 1995; Morton and Faust, 1997; Parsons et al., 2011; Tester et al., 2014). It is hypothesised that *Gambierdiscus* spp. are inadvertently consumed by herbivorous fish grazing on macroalgae (Heimann et al., 2011; Randall, 1958). Environmental disturbances such as coral bleaching, cyclones and storms impact coral reefs and are often followed by increases in macroalgal cover (Diaz-Pulido et al., 2009). The duration of macroalgal dominance on reefs may be exacerbated by predicted climate change and other anthropogenic disturbances including pollution and overfishing (Hughes et al., 2010; Welsh and Bellwood, 2015).

The warmer sea surface temperatures (SSTs) associated with increased ciguatera incidence (Hales et al., 1999; Llewellyn, 2010; Tester et al., 2010) are within the range of temperatures experimentally documented for optimal growth of Caribbean and Pacific strains of *Gambierdiscus* species (Kibler et al., 2012). Warmer SSTs and reduced salinities are primary triggers of coral bleaching (Hoegh-Guldberg, 1999; Hoegh-Guldberg and Bruno, 2010). Temperature-triggered coral bleaching induces the loss of endosymbiotic dinoflagellates, which results in pigment loss in corals compared to loss of coral tissue due to coral bleaching triggered by reduced salinity (Kerswell and Jones, 2003). Substantial freshwater inputs from storms and cyclones can produce short-lived pockets of reduced salinities in shallow, protected areas on reefs (Hoegh-Guldberg and Smith, 1989); while reduced salinities associated with flood plumes impact coastal and inshore reefs (Kerswell and Jones, 2003). Flood

plumes generated by cyclones and storms during wet seasons on the Great Barrier Reef (GBR) have been reported 150 – 200 km offshore, where lowered salinities between 26 to <10 may be short lived over a few days or persist for up to three weeks (Devlin et al., 2001). It is also hypothesised that the spatial magnitude of coral bleaching events triggered by warmer SSTs is influenced by the strength of El Niño periods (Hoegh-Guldberg, 1999). The degree of mortality caused by coral bleaching dictates the duration of macroalgal dominance (Graham et al., 2015). Macroalgal outbreaks and benthic phase shifts on coral reefs may be an important facilitator in the growth and spatial expansion of *Gambierdiscus*, and thus, potentially, ciguatera outbreaks.

The duration of macroalgal dominance is also influenced by the frequency, severity and combined effects of environmental disturbances. Jamaican reefs in the Caribbean have experienced long-term macroalgal dominance following the combined effects of hurricanes, overfishing and the mass die off of the grazing sea urchin *Diadema antillarum* (Hughes, 1994). Subsequent increases in benthic dinoflagellate populations, including *Gambierdiscus* spp. have been observed, which may increase the occurrence of ciguatera (Chateau-Degat et al., 2005), but no ciguatera cases were reported following a bloom of *Gambierdiscus toxicus* subsequent to coral bleaching at the Mayotte reefs in the Indian Ocean (Turquet et al., 2001). Indeed, drivers of toxin production have yet to be determined for known toxic *Gambierdiscus* spp. Although high concentrations of ciguatoxins and maitotoxins can build up in cultures over long time frames, the typical low or undetectable concentrations of toxin produced by cultured *Gambierdiscus* cells in short-duration experiments limits exploration of

the effect of transient environmental disturbances and key ecological drivers on toxin production, negatively affecting prediction of risk of ciguatera incidence.

Temperature and salinity were identified as potential key ecological drivers in the population growth of *Gambierdiscus* by Kibler et al. (2012) who established that tolerance ranges varied between eight *Gambierdiscus* species. This may reflect local thermal adaptation, as Caribbean species preferred warmer temperatures compared to temperate species from North Carolina, USA. In Australia, *Gambierdiscus carpenteri* and co-occurring benthic toxic dinoflagellates have been found at locations throughout the GBR (Murray et al., 2014). There is, however, little understanding of the response of endemic *Gambierdiscus* populations to temperature and salinity changes that are commonly experienced on reefs in the GBR.

Ocean currents have been a primary vector route for reef fish and other marine species from tropical regions to warm temperate marine habitats (Booth et al., 2007). Fish larvae have been transported from the tropics northwards into temperate regions by the Gulf Stream in North America (Kibler et al., 2015). In Australia, tropical species have been transported to temperate marine habitats by the Leeuwin Current along the west coast and from the GBR on the east coast by the East Australian Current (EAC) (Booth et al., 2007; Figueira and Booth, 2010). Seasonal occurrences of juvenile tropical fish has been observed off the New South Wales (NSW) coast for more than a decade (Figueira et al., 2009). Strengthening of the EAC during the Austral summer promotes the

seasonal transport of tropical fish into temperate waters as far south as Merimbula (36°S, 149°E) in southern NSW (Figueira and Booth, 2010). Recent records of *G. carpenteri* and co-occurring benthic toxic dinoflagellates in Merimbula also suggests that the EAC was a likely primary transport mechanism from GBR tropical waters (Heimann et al., 2011; Kohli et al., 2014; Murray et al., 2014). In comparison to the dominance of coral reefs in tropical regions, rocky coastlines and macroalgal forests dominate temperate marine habitats, which may provide suitable substrates for colonisation by benthic toxic dinoflagellates. The current expansion of bio-geographic boundaries for ciguatera-causing dinoflagellates is likely to continue under predicted climate developments (Heimann et al., 2011; Kibler et al., 2015; Tester et al., 2013). It is therefore essential to understand temperature and salinity tolerances, as these are drivers governing the distribution and abundance of ciguatera-causing dinoflagellates. This may be critical in the evaluation of temperate marine fish species likely to be at higher ciguateric risk.

A positive correlation between SSTs and population size of *Gambierdiscus* spp. have been demonstrated consistently over decades (Bomber et al., 1988a; Chinain et al., 1999b; Kibler et al., 2012; Llewellyn, 2010). In 1994, larger populations of *Gambierdiscus* spp. were recorded seven months after temperature-triggered coral bleaching in Tahiti, French Polynesia; these higher cell densities continued to be recorded for 24 months following the bleaching event (Chinain et al., 1999b). Lewis et al. (1986) reported a decrease in *Gambierdiscus* populations three months after a cyclone over Sudbury Reef, off Cairns, Australia. Colonization of dead coral substrates from coral bleaching or

cyclones have been reported to occur through a succession of macroalgal species (Cheal et al., 2010; Diaz-Pulido and McCook, 2002b; Hughes et al., 2007). Initial colonization by filamentous algal turfs generally occurred within the first month in the GBR, French Polynesia and the Indian Ocean following an environmental disturbance (Bagnis and Rougerie, 1992; Diaz-Pulido and McCook, 2002b; Turquet et al., 2001; Walsh, 1983). Surveys in the Society Archipelago, French Polynesia and Mayotte, Indian Ocean reported the subsequent increase in *Gambierdiscus* populations (>150-fold higher (60,463 cells g^{-1} algae at Mayotte)) in the two – three months following, (Bagnis and Rougerie, 1992; Turquet et al., 2001). The influence of environmental drivers that trigger population increases of *Gambierdiscus* spp. and co-occurring benthic dinoflagellates on coral reefs remain, however, contradictory. Growth studies on *Gambierdiscus* by Morton et al. (1992) showed preference for lower salinities, which contrasted with studies by Kibler et al. (2012) reporting reduced growth at salinities lower than 20. Lower salinities during the wet season were speculated to affect seasonality in *Gambierdiscus* populations (Bomber et al., 1988a; Gillespie et al., 1985), however, the interaction of salinity and temperature remains unexplored.

Stable co-existence of *Gambierdiscus* spp., *Ostreopsis* spp. and *Prorocentrum* spp. could be a result of positive interspecific interactions, such as allelopathy. Early laboratory studies established allelopathic interactions in benthic dinoflagellates through supplementing culture media of one species with filtered exudate from another species and in bi-algal cultures (Bomber, 1990). As in planktonic dinoflagellates, positive allelopathic interactions are thought to favour

benthic dinoflagellates by deterring grazing and improving nutrient resource allocation (García-Portela et al., 2016; Gross, 2003). Inhibitory effects on growth using filtered-exudates have been documented for *G. toxicus, Prorocentrum* spp., *Ostreopsis* spp. and *Coolia monotis* (García-Portela et al., 2016; Monti and Cecchin, 2012; Sugg and VanDolah, 1999). Growth of co-occurring diatoms were also inhibited by *G. toxicus* (Bomber, 1990), however, Monti and Cecchin (2012) reported that the filtrate from *O. ovata* had no effect on the growth of the diatom, *Coscinodiscus granii*. Although inhibitory effects were most frequently observed, Bomber (1990) reported stimulatory effects between species of *Prorocentrum*. Interestingly, although benthic dinoflagellates frequently associate with macroalgal substrates, Accoroni et al. (2015) documented inhibitory effects on the growth of *O. ovata* in bi-algal cultures and filtrates from three macroalgae, *Dictyota dichotoma, Ulva rigida* and *Rhodymenia pseudopalmata*. The few studies that have reported allelopathic experiments with benthic dinoflagellates were conducted in culture conditions, where temperature and salinity were constant. Kibler et al. (2012), however, reported broad temperature (15-33ºC) and salinity (<14 – >41) tolerances for eight species of *Gambierdiscus* potentially reflecting their biogeographic origins. The impact of lower salinities and warmer temperatures on interspecific interactions, such as allelopathy in mixed benthic dinoflagellate assemblages have not been previously explored.

This study aimed to examine the interaction of temperature and salinity on the population growth of two strains of *G. carpenteri*, isolated from an inshore location of the central Great Barrier Reef and from the waters of the ReefHQ

aquarium, Townsville. It was hypothesised that populations of the inshore *G. carpenteri* strain would have higher growth rates at lower temperatures and salinities than the *G. carpenteri* strain isolated from the regulated aquarium environment. Fluctuation of inshore environmental stressors should also affect assemblage structure of benthic dinoflagellates. Therefore, this study also aimed to investigate the potential interspecific interactions of a mixed assemblage consisting of *P. lima* and *Ostreopsis* sp. at different salinities on the population growth of *G. carpenteri*, NQAIF 116 isolated from an inshore habitat at Pallarenda, Townsville. Simulation of likely adverse environmental conditions in experiments with mixed assemblages will improve our understanding of co-habiting benthic dinoflagellate responses. This will also provide an insight into key ecological drivers for population growth and capacity for range expansion.

5.3 Methods

5.3.1 Cell cultures

The cultures of *Gambierdiscus carpenteri* NQAIF116, was established from a sample collected on macroalgae at the mouth of Three Mile Creek, Pallarenda (19°S, 146°E), in the central Great Barrier Reef, (Murray et al., 2014) Australia. Cultures of *Gambierdiscus* sp. (NQAIF380, hereafter identified as *G. carpenteri*), *Prorocentrum lima* (NQAIF379) and *Ostreopsis* sp. (NQAIF382) were established from water samples collected in the coral reef exhibit at ReefHQ aquarium, Townsville. All cultures were isolated and established at the North Queensland Algal Identification and Culturing Facility (NQAIF) at James Cook University, Townsville, Australia by the collection curator, Mr. Stanley

Hudson. Cultures were maintained in K medium at 24°C and a salinity of 36 in a temperature and light controlled Contherm phytoplankton growth chamber with a 12:12 hour light:dark cycle. Cultures were acclimated at 28°C over a period of eight days (equivalent to two cell divisions for *Gambierdiscus* species) prior to inoculation for treatments at 28 and 34°C. To simulate changes in salinity on reefs in the Austral wet season (see introduction), cultures were not acclimated to lower salinities prior to treatments.

5.3.2 Culture identification

Both cultures (NQAIF116 and NQAIF380) were unambiguously identified as strains of *G. carpenteri*. The culture NQAIF116 was characterized by Murray et al. (2014) using both calcofluor white staining and epifluorescence microscopy (Murray et al., 2014) and via phylogenetic reconstruction using the near complete 18S rRNA gene and the D8-D10 region of the 28S rRNA gene.

To determine the identity of the second strain (NQAIF380), we obtained the D8-D10 region of the 28S rRNA gene and constructed a phylogeny using a subsample of 37 sequences employed by Murray et al. (2014) representing 15 clades of the genus *Gambierdiscus sensu lato—i.e* including the globular species which have recently been moved to the genus *Fukuyoa* (Gómez et al., 2015). Deoxyribose nucleic acid (DNA) was extracted using a modified Chelex® protocol (Walsh et al., 1991) as outlined in Momigliano et al. (2013). A 929 bp long fragment of DNA including the D8/D10 region of the 28S rRNA gene was amplified using the primer pair FD8 and RB (Chinain et al., 1999a), using the same PCR conditions as per Murray et al. (2014). The amplified fragment was sequenced with the FD8 and RB primers by a commercial service (Macrogen

Inc. Seoul, Korea). A maximum likelihood tree was estimated using the software PHYML 3.1 (Guindon et al., 2010), using five random starting trees and tree improvement using the best of nearest neighbour interchange (NNI) and subtree pruning and regrafting (NPR) for tree improvement. We used the substitution model TrN+G with four gamma categories (alpha=0.46), which was selected using the software jModelTest2 (Darriba et al., 2012). Branch support was estimated by analysing 1000 bootstrap datasets.

5.3.3 Experimental treatments

To investigate the effect of temperature and salinity on population growth for two strains of *Gambierdiscus carpenteri* (NQAIF116 and NQAIF380), a factorial experimental design was chosen. Four replicate 24-well tissue culture plates were prepared at each temperature (24, 28 and 34°C) and for each salinity (16, 26 and 36), eight wells per plate were randomly selected (n=32 per treatment). In analogy to tissue culture approaches and to methods outlined in Holland et al. (2013), each well of the 24-well tissue culture plates contained 2mL K medium (without silicate), and each well was inoculated with groups of 4-10 cells of each *G. carpenteri* strain (as growth for *G. carpenteri* was inconsistent with <4 cells per well), using the capillary capturing technique on an inverted microscope (Olympus CKX41, Olympus, Sydney, Australia) at x 200 final magnification.

The second experimental treatment was designed to investigate the hypothesis that a mixed assemblage of benthic dinoflagellates has a positive effect on population growth of *G. carpenteri* from inshore habitats, which experience

naturally fluctuating salinities. To investigate the effect of salinity and a mixed assemblage of benthic dinoflagellates on the growth of inshore *G. carpenteri* strain NQAIF116, four replicate 24-well tissue culture plates were prepared at 24°C and for each salinity (16, 26 and 36), three wells per plate were randomly selected. Controls (wells with monoculture of inshore *G. carpenteri* strain NQAIF116) and treatments (NQAIF116 in mixed assemblages of *P. lima*, NQAIF379 and *Ostreopsis* sp. NQAIF382) were prepared to demonstrate an effect of a mixed assemblage on the growth of NQAIF116. To investigate the potential effect of a dominant dinoflagellate in mixed assemblages, the treatment was further partitioned to *P. lima*- or *Ostreopsis* sp.-dominated assemblages. As described above, the wells in each of the four replicate 24-well tissue culture plates contained 2mL K medium (without silicate) and were inoculated using the capillary capturing technique on the inverted microscope at x 200 final magnification. Cell densities at inoculation varied and the mean number of cells per dinoflagellate showed that in the treatments, inoculation proportions favoured *P. lima* NQAIF379 (Table 5.1).

Table 5.1 The mean inoculation cell densities for mixed assemblage experiments with control (monoculture of NQAIF116) and treatment (mixed assemblage of NQAIF116, NQAIF379 and NQAIF382)

	Control			Treatment		
Salinity	**36**	**26**	**16**	**36**	**26**	**16**
***Gambierdiscus carpenteri* NQAIF116**	10	12	10	8	9	7
***Prorocentrum lima* NQAIF379**				22	22	30
***Ostreopsis* sp. NQAIF382**				5	5	6

For all experimental treatments, direct cell counts of the small aggregates of the entire well were used to measure population growth for all dinoflagellate

populations. Cell aggregates typically did not exceed 50-60 cells/ aggregate. Counts were conducted on day zero (one day after inoculation) and every fourth day over a period of 28 days on an inverted microscope at 40x final magnification for *Gambierdiscus* species and at 200x final magnification for *P. lima* and *Ostreopsis* sp. As mixed assemblage experiments investigated the cell to cell effect similar to García-Portela et al. (2016) on the population growth *G. carpenteri* NQAIF116, cell numbers for *P. lima* and *Ostreopsis* sp., with and without *G. carpenteri* NQAIF116, were converted to relative abundance (% contribution to assemblage). Cell counts were conducted until day 8 or after cells exceeded 250, and then relative abundance was estimated. In addition, 24-well tissue culture plates were sealed with parafilm to avoid salinity changes due to evaporative water loss among wells on a culture plate. Salinity was checked in randomly selected wells at the end of the experiment to verify nominal salinities were retained. Relative population growth rate (*r*) and doubling time (*k*) were determined using formulae described in (Wood et al., 2005b).

5.3.4 Statistical Analysis

The effects of treatments (temperature, salinity) on population growth of *G. carpenteri* over the 28-day period was assessed by a two-way ANOVA with an orthogonal factorial design. To investigate the effect of temperature and salinity on growth of NQAIF116 and NQAIF380, the experimental design had nine treatments (3 temperatures, 3 salinities), each with a total of 32 replicate wells per treatment (8 replicate wells per salinity on each of the four 24-well plates at each temperature). An experimental design with 12 replicate wells (3 replicates per plate per treatment on four 24-well plates each) per treatment was used to

investigate the effect of a mixed assemblage of benthic dinoflagellates (*P. lima* and *Ostreopsis* sp.) and salinity on NQAIF116. Statistical results are presented in the supplementary materials Table S5.1, Appendix E.

5.4 Results

5.4.1 Culture identification

A 929 fragment of the D8-D10 region of the 28S rRNA gene was obtained from culture NQAIF380. This sequence was identical to the sequence obtained by Murray et al (2014) from the strain of *G. carpenteri*, NQAIF116. The strain grouped with 100% bootstrap support within the *G. carpenteri* clade, providing unambiguous species-level identification (Supplementary Fig. S5.1, Appendix F).

5.4.2 Effects of temperature and salinity

Population growth of the inshore *G. carpenteri* NQAIF116 strain was near identical for 24 and 28°C at salinities of 36 and 26 (Fig. 5.1 A-B), while *G. carpenteri* NQAIF380 isolated from the environmentally stable ReefHQ coral reef exhibit, showed higher growth at 28°C at these two salinities (Fig. 5.1 D-E). Both strains, NQAIF116 and NQAIF380, had negative growth at a temperature of 34°C (all salinities) and at a salinity of 16 (all temperatures) (Fig. 5.1 A-F, Table 5.2). Both strains had highest mean population growth rates and cell divisions day^{-1} in salinities of 36 at temperatures of 24 and 28°C (Table 5.2) and *G. carpenteri* NQAIF380 had the highest average cell count of 417 cells/well on day 28 at 28°C (Fig. 5.1D). There was, however, no significant interaction between the effect of temperature and salinity when growth was positive (ANOVA $p>0.05$).

Population growth rates and cell divisions day^{-1} for NQAIF116 were higher compared to NQAIF380 at 24°C at salinities of 36 and 26 (Table 5.2). Mortality occurred at 34°C for *G. carpenteri* NQAIF116 populations on day 12 at salinities of 36 and 26. At a salinity of 16, however, populations of inshore NQAIF116 strain stabilised by day 28 (Fig. 5.1C), suggestive of potential recovery.

Temperature and salinity had a significant effect on the positive mean population growth of *G. carpenteri* strain, NQAIF380 (ANOVA $_{df1, 124}$ $p<0.05$) at 24 and 28°C (Fig. 5.1D-E). This contrasted with the inshore *G. carpenteri* strain, NQAIF116 where temperature did not have a significant effect on positive growth (ANOVA $_{df1, 124}$ $p>0.05$). Population growth rates and cell divisions.day^{-1} for NQAIF380 were higher at 28°C compared to 24°C at salinities of 36 and 26 (Table 5.2). At a salinity of 16, NQAIF380 populations at 34°C survived until day 16, which was 4-8 days longer than for populations maintained at salinities of 26 and 36 (Fig. 5.1D-F).

Table 5.2 Population growth rate (*r*) and divisions.day^{-1} (*k*) for *Gambierdiscus carpenteri* NQAIF116 and NQAIF380 at 24, 28 and 34°C and in the mixed assemblage (NQAIF116+) at 24°C in 36, 26 and 16 salinity.

		NQAIF116		NQAIF380		NQAIF116+	
°C	**Salinity**	***r***	***k***	***r***	***k***	***r***	***k***
	36	0.11 ±0.01	0.17 ±0.02	0.08 ±0.00	0.11 ±0.01	0.09 ±0.01	0.13 ±0.01
24	**26**	0.07 ±0.02	0.10 ±0.03	0.01 ±0.01	0.02 ±0.01	0.11 ±0.01	0.16 ±0.01
	16	-0.03 ±0.01	-0.04 ±0.01	-0.03 ±0.01	-0.04 ±0.01	-0.02 ±0.02	-0.02 ±0.03
	36	0.10 ±0.01	0.14 ±0.01	0.12 ±0.01	0.17 ±0.02		
28	**26**	0.06 ±0.01	0.09 ±0.02	0.07 ±0.02	0.10 ±0.03		
	16	-0.04 ±0.00	-0.05 ±0.00	-0.02 ±0.02	-0.03 ±0.03		
	36	-0.03 ±0.02	-0.05 ±0.02	-0.02 ±0.01	-0.03 ±0.01		
34	**26**	-0.05 ±0.01	-0.07 ±0.01	-0.01 ±0.01	-0.01 ±0.01		
	16	-0.03 ±0.00	-0.05 ±0.01	-0.01 ±0.00	-0.02 ±0.01		

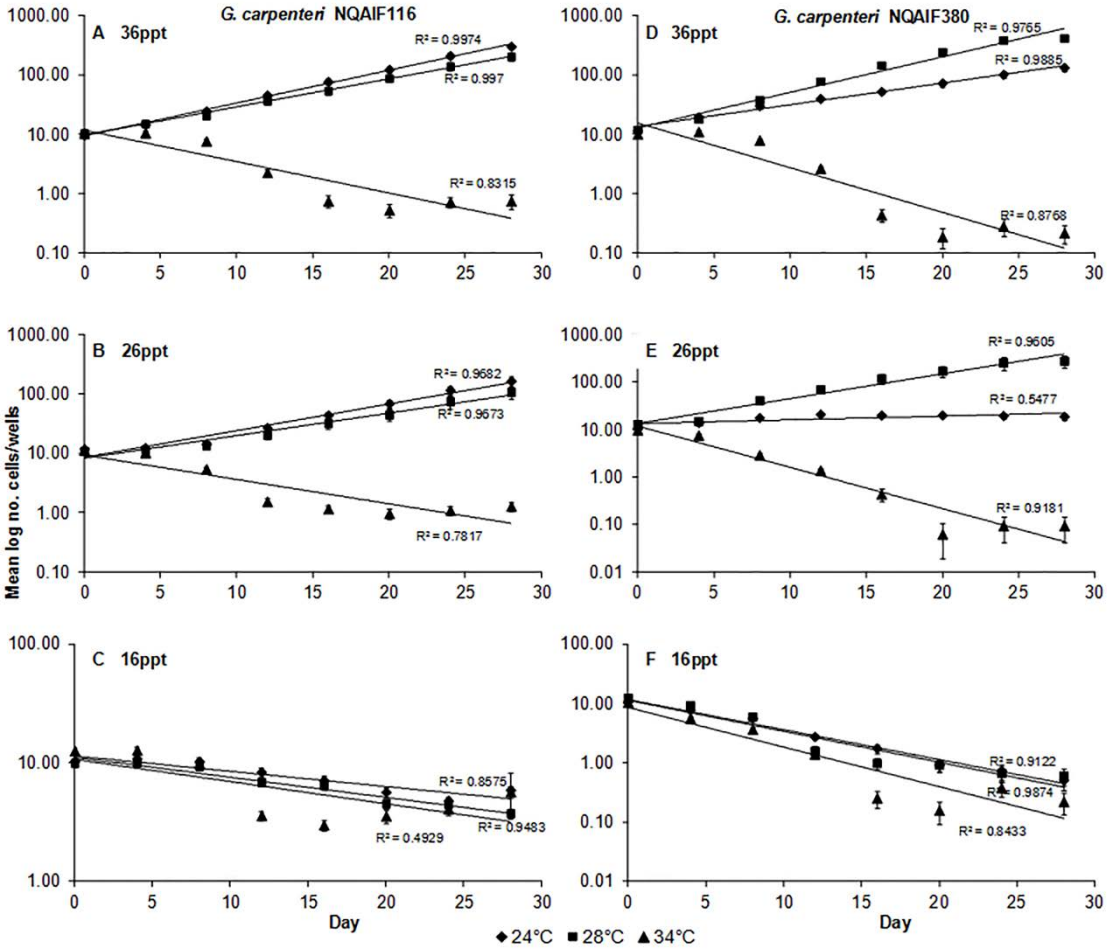

Figure 5.1 Effect of temperature and salinity on the growth of two strains of *Gambierdiscus carpenteri*. Growth is shown as the mean number of cells/well (±SE), n = 32. A-C: NQAIF116 at 36 salinity (A), 26 salinity (B) and 16 salinity (C) and D-F: NQAIF380 at 36 salinity (D), 26 salinity (E) and 16 salinity (F). Note, error bars that are not visible do not exceed size of the symbols. Regression lines for the time-series data are shown.

5.4.3 Effect of mixed dinoflagellate assemblages on the growth of inshore strain of *G. carpenteri,* NQAIF116

Salinity and presence of a mixed assemblage had a significant effect on the growth of *G. carpenteri* NQAIF116 ($ANOVA_{df\ 1,\ 66}$ $p<0.05$). Mean population growth of NQAIF116 at a salinity of 36 was inhibited by the presence of a mixed assemblage of dinoflagellates (Fig. 5.2A), irrespective of assembly dominance, with inhibition strongest in *P. lima*-dominated assemblages (Fig. 5.2A). At a

salinity of 26, presence of the mixed assemblage had no measurable effect on the growth of *G. carpenteri* (Fig. 5.2B), but the relative population growth rate and divisions.day^{-1} of NQAIF116 increased in a mixed assemblage (Table 5.2). Growth of *G. carpenteri* was severely reduced at a salinity of 16 in both the presence and absence of the mixed assemblage (Fig. 5.2C), but *G. carpenteri* showed a ~80-fold increase in growth in the presence of *Ostreopsis*-dominated assemblages, while *Prorocentrum*-dominated assemblages did not improve growth performance (Fig. 5.2C).

5.5 Discussion

On the east coast of Australia, ciguatera is prevalent on the Great Barrier Reef (GBR) (Lewis, 2006). Under predicted climate change scenarios, occurrence of coral bleaching events and risk of ciguatera are anticipated to increase with warmer SSTs (Llewellyn, 2010; Walther et al., 2002). The causative organisms of ciguatera, *Gambierdiscus* spp. have recently been documented throughout the GBR, with *G. carpenteri* (NQAIF116) occurring in the central GBR (Murray et al., 2014). Freshwater influxes from storms, cyclones and flood plumes affect coastal habitats and inshore coral reefs by causing changes in temperature and salinity within the ranges chosen for this study. Currently, the genus of *Gambierdiscus* is experiencing reclassification and new species are being identified (Fraga et al., 2016; Kretzschmar et al., 2017; Smith et al., 2016). To date, 16 species of *Gambierdiscus* (includes new species and *G. yasumotoi*, which has been moved to a new genus *Fukuyoa*) have been identified globally, with species demonstrating a variable response to environmental stressors,

such as temperature, salinity, and light (Kibler et al., 2012). In our experimental design,

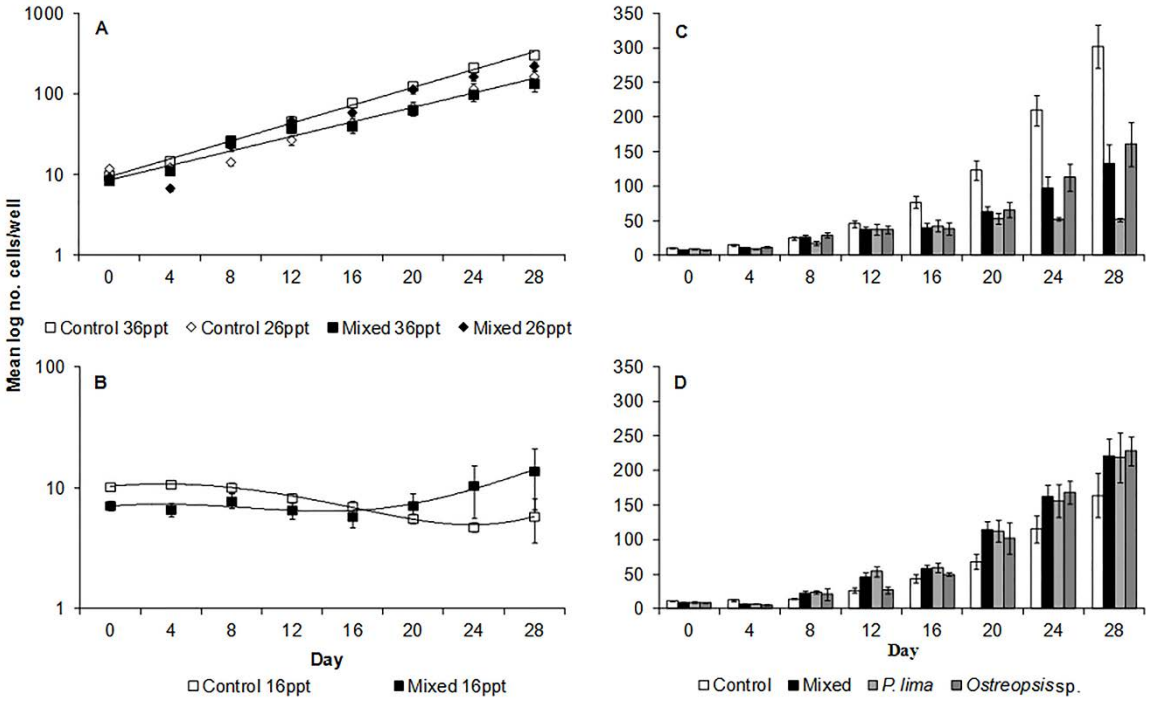

Figure 5.2 The effect of mixed benthic toxic dinoflagellate assemblage dominance on the growth of *Gambierdiscus carpenteri* (NQAIF116) at different salinities and 24 °C. Growth is shown as the mean number of cells/well (±SE), n = 32. Growth of *G. carpenteri* was measured with *P. lima*-dominated and *Ostreopsis*-dominated mixed assemblages and in *Gambierdiscus* monocultures (control).

mother cultures were maintained at 24 °C, and acclimated to 28 °C for eight days, prior to inoculation for 28 and 34 °C. So a temperature shift of a maximum of 6 °C was experienced by the 34 °C and 4 °C for the 28 °C experimental cultures. In shallow protected reef environments, where these benthic dinoflagellates occur, temperature shifts of this magnitude are readily observed to occur over just a day or two, typically resulting in bleaching responses of corals. As expected for an organism isolated from an inshore environment, population growth of *G. carpenteri* NQAIF116 was comparable at 28 and 24°C for salinities of 36 and 26. At a salinity of 16, several cells survived at all three temperatures, but no increase in population size was observed. This response

to the different combinations of temperature and salinity scenarios suggests that NQAIF116 is tolerant to prevalent coastal conditions and has the potential to colonize temporary macroalgal substrates that often follow coral bleaching events (Cheal et al., 2010; Johns et al., 2014). The adaptability to a broad range of habitat conditions is thought to contribute to the global distribution of this species (Xu et al., 2016). It is these characteristics that would make NQAIF116 a likely candidate for range expansions southward on the East Australian coast into warm-temperate regions, such as those documented in the seasonal occurrence of *G. carpenteri* populations in southern NSW, Australia (Kohli et al., 2014). Similar to *Symbiodinium* sp. isolated from a mid-reef coral at Heron Island, which did not survive at 32°C (Rosic et al., 2011), *G. carpenteri* NQAIF380 did not survive 34°C, irrespective of salinity.

During tropical wet seasons, freshwater influxes frequently occur inshore, but occasionally extend to shallow coral reefs and cays up to 200 km from shore, with plumes persisting for up to 3 weeks (Devlin et al., 2001). Freshwater influxes from rivers due to cyclones and storms can lower salinities to the levels used here (Hoegh-Guldberg and Smith, 1989). Populations of *G. carpenteri* NQAIF380 isolated from an environmentally controlled coral reef aquarium exhibit of ReefHQ, Townsville, Australia showed tolerance to a decline in salinity from 36 to 26 at 28°C only, which suggests a narrow temperature optimum, suggestive of less variable temperatures and salinities often associated with mid- and outer-shelf GBR reefs (Fallon et al., 2003). Taking 28°C and a salinity of 26 as conditions often encountered in estuarine environments, growth rates of both *G. carpenteri* strains were comparable and

population sizes were smaller than at a salinity of 36 at the same temperature. This supports the scenario proposed by Kibler et al. (2015) of smaller *G. carpenteri* populations in coastal environments with variable environmental conditions, such as in mangroves and estuaries. Compared to the steady decline of NQAIF380 populations at a salinity of 16, NQAIF116 appeared to stabilise with time at this salinity at all three temperatures, including 34°C. Different responses to temperature and salinity changes shown by the two strains of *G. carpenteri* may be indicative of local adaptation to different environmental conditions. Xu et al. (2016) suggested that geographic origin influences tolerance to environmental parameters, as *Gambierdiscus carolinianus* that originated from North Carolina tolerated lower temperatures compared to a *G. carolinianus* strain from St Thomas, U.S. Virgin Islands. Both strains of *G. carpenteri* in this study were isolated from a similar latitude in the central GBR, however, each showed different responses to the environmental parameters. This suggests that, in addition to the influence of geographic origin suggested by Xu et al. (2016), the local environmental conditions of the original habitat from which the strains were isolated, i.e. estuaries, inshore reefs, or mouth or rivers and creeks, such as *G. carpenteri*, NQAIF116, can also influence their physiological acclimation potential, tolerance and population growth. Low growth in response to environmental stressors, such as warmer temperatures and changes in salinity can be explained by higher metabolic costs and potential for photoinhibition (Rosic et al., 2011). Changes in salinity are likely to initiate osmolyte production (von Alvensleben et al., 2016) and fine-tuning of expression of various heat shock proteins (HSPs) (Rosic et al., 2011), with both survival strategies incurring metabolic cost (Kibler et al., 2012). For

example, reduced salinity could cause a cell's internal K^+/Na^+ pump to be more frequently engaged to modify cell size and/or turgor (von Alvensleben et al., 2016). Variable expression of HSPs has been well studied in corals due to concerns over consequences of increased SSTs (Rosic et al., 2011). Regulation of HSP expression patterns have been described for a variety of environmental stressors (Rosic et al., 2011). Increased production of Hsp70 at 26 and 29°C was shown for the endosymbiotic dinoflagellate, *Symbiodinium* sp. (isolated from the coral *Acropora millepora* at Heron Island, GBR), but levels decreased at 32°C. In contrast, Hsp90 was down-regulated at all warmer temperatures. Rosic et al. (2011) reported genetic similarity with other dinoflagellates for Hsp70, including *Prorocentrum minimum*, and for Hsp90, including *Alexandrium tamarense*. It is likely that HSPs may also have an important role in the response of benthic dinoflagellates to local environmental stressors, such as freshwater plumes, river runoff and coral bleaching events. It is suggested that the down-regulation of Hsp90 is important for organisms to acclimate to local and variable environmental conditions (Rosic et al., 2011). In our study, *G. carpenteri* NQAIF116 showed similar population growth rates between 36 and 26 salinities at 24 and 28°C, as well as strong decline at 34°C but not complete mortality even at a salinity of 16. It would be very interesting to profile HSP expression patterns in response to salinity and temperature changes, with both stressors in isolation as well as combined (the latter will more closely resemble natural conditions).

Temperature has been identified as a key driver of population size and distribution of *Gambierdiscus* populations (Chateau-Degat et al., 2005; Kibler et

al., 2015). Fluctuations in salinity from freshwater inputs, however, often co-occur with high temperatures that trigger coral bleaching. The GBR spans more than 12° latitude with a thermal latitudinal gradient documented for corals (Berkelmans, 2002) and foraminifera (Momigliano and Uthicke, 2013). Recent studies have shown a thermal latitudinal gradient of tolerance in some *Gambierdiscus* species in the Caribbean to North Carolina, USA (Kibler et al., 2012; Xu et al., 2016). Further investigations are required to understand the impact of climate change scenarios on the distribution and abundance of *Gambierdiscus* species on the GBR and for revealing the existence of thermal latitudinal gradients of tolerance on distributions of *G. carpenteri* and other *Gambierdiscus* species on the GBR.

Species of *Gambierdiscus* are typically found on macroalgal substrates as part of the benthic dinoflagellate assemblage, which is often dominated by *Prorocentrum* spp and *Ostreopsis* spp (Morton and Faust, 1997). There have only been a few studies that investigated interspecific interactions, such as allelopathy on population growth of benthic dinoflagellates. Inhibitory effects were reported between co-occurring benthic dinoflagellates, including inhibition of *G. toxicus* by cell-free filtrates and in bi-algal cultures with *Prorocentrum* spp. or *Ostreopsis* spp. (Bomber, 1990; García-Portela et al., 2016; Sugg and VanDolah, 1999). The contribution of interspecific interactions on the abundance and distribution of *Gambierdiscus* spp. under environmental stress, such as lower salinities from freshwater plumes or heavy rains from cyclones, have not been previously investigated. This study observed that a mixed assemblage of *P. lima* and *Ostreopsis* sp. also had an inhibitory effect on the

population growth of *G. carpenteri* NQAIF116 at 24°C at a salinity of 36, with the strongest inhibitory effect elicited by *P. lima*-dominated assemblages, while at a salinity of 26 there was no significant effect. At a salinity of 16, overall growth rate was reduced substantially, but *Ostreopsis*-dominated assemblages showed increased growth of *G. carpenteri* NQAIF116. Our results are not in accordance with suggestions raised in some other studies suggesting that allelochemicals are produced under environmental stress to outcompete other organisms for resources (Monti and Cecchin, 2012; Sugg and VanDolah, 1999). Interestingly in this context, cell densities affected allelopathic outcomes in mixed populations of the dinoflagellate *Scrippsiella trochoidea*, with population growth stimulated by cell-free filtrates of *Prorocentrum donghaiense* at low cell densities ($1.9x10^4$ cells mL^{-1}) but inhibited by filtrates obtained from high cell densities cultures ($1.9x10^5$ cell mL^{-1}) (Wang and Tang, 2008). This suggests that cell densities of benthic dinoflagellates in a mixed assemblage can significantly impact on allelopathic outcomes for *Gambierdiscus* and our data suggest that under stressful conditions *Ostreopsis* sp.-dominated assemblages may improve tolerance of *Gambierdiscus* spp. to environmental stressors, such as lower salinities. Further ecological studies are, however, needed to understand the reasoning for the frequent occurrence of mixed benthic dinoflagellate assemblages when inhibitory effects have most often been reported. Dominance profiles in mixed benthic dinoflagellate assemblages on inshore reefs, central GBR are variable. *Ostreopsis* spp. dominated mixed assemblages at Nelly Bay, Magnetic Island (19°S, 146°E), however, *Prorocentrum* spp. were dominant within mixed assemblages of inshore reefs on Orpheus and Pelorus Islands (18°S, 146°E) (Skinner et al., 2013; Sparrow

and Heimann, 2016). In line with this laboratory study, generally lower numbers of *Gambierdiscus* spp. were observed on inshore reefs at Orpheus and Pelorus Island compared to Nelly Bay (Sparrow and Heimann, 2016). The results suggest that environmental stressors, such as salinity and temperature, as well as the dominant dinoflagellate species in mixed benthic dinoflagellate assemblages, may affect growth and/or survival of *Gambierdiscus* spp. populations on inshore reefs in the central GBR. This highlights the interaction between identity and abundance of benthic toxic dinoflagellates as a potential key driver in the distribution and abundance of *Gambierdiscus* populations. Accoroni et al. (2015) recently documented allelopathic interactions between *Ostreopsis ovata* and species of macroalgae, which implies that allelopathic interactions may also influence the suitability of macroalgal substrates for benthic toxic dinoflagellates and thus could potentially affect geographic expansion of such dinoflagellates. Further exploration into interspecies interactions, such as allelopathy between benthic dinoflagellates and their macroalgal substrates may identify causal factors in substrate preferences.

In summary, this study shows that prior environmental history of *G. carpenteri* strains influences temperature and salinity tolerances, with NQAIF116 originating from a highly variable environment being more tolerant than NQAIF380, isolated from a controlled aquarium environment. This has implications for the potential of range expansions under predicted climate change scenarios and potential spread of ciguatera beyond its hitherto known natural boundaries. Furthermore, dominance profiles of mixed assemblages affected growth of *G. carpenteri* NQAIF116 at 24°C with *P. lima*-dominated

assemblages having a stronger inhibitory effect at all salinities. In contrast *Ostreopsis*-dominated mixed assemblages rescued growth of *G. carpenteri* NQAIF116 at a salinity of 16. The mixed assembly results indicate that interspecies interactions change under environmental stress and this information should be integrated into future studies of allelopathic interactions in benthic toxic dinoflagellate assemblages.

Chapter Six: Effect of temperature and salinity on growth, nutrient uptake and biochemical profile of benthic dinoflagellates isolated from the central Great Barrier Reef

6.1 Abstract

Ciguatera, tropical reef fish poisoning, is the most commonly reported seafood-associated illness and is caused by ciguatoxin-producing dinoflagellates *Gambierdiscus* spp. through bioaccumulation and bioconversion of the toxins in marine food webs. *Gambierdiscus* spp. are found together with the benthic toxin-producing dinoflagellates *Prorocentrum* and *Ostreopsis*. Recently, populations of *G. carpenteri* have been recorded from coastal waters in New South Wales, Australia, representing a substantial range expansion southward. The aim of this study was to determine the effect of lower temperature and salinity on population growth, nutrient uptake rates and fatty acid profiles of *G. carpenteri*, *P. lima* and *Ostreopsis* sp. isolated from the Great Barrier Reef (GBR). Cultures were grown at 24 and 28 °C and at salinities of 36 and 26 over a period of 28 days. To maximise biomass for fatty acid analysis, cultures were kept nutrient-replete. Population growth and nutrient uptake rates of *G. carpenteri* were reduced significantly at a salinity of 26 at both temperatures. Nutrient uptake rates increased for *P. lima* at the lower salinity at 28 °C, with no effect of temperature or salinity on population growth. By contrast, low temperature (24 °C) reduced population growth but increased nutrient uptake in *Ostreopsis* sp. at both salinities investigated. The effect of reduced temperature

and salinity on fatty acid profiles, indicators of food quality, was investigated. A principal component analysis showed separation of species based on the sum of saturated fatty acids and polyunsaturated fatty acids (Σ PUFA). Low salinity (26) was positively associated with Σ PUFA for *Ostreopsis* sp., but Σ PUFA content was lowest at a salinity of 26 at 24 °C for *G. carpenteri*. By contrast, fatty acid profiles for *P. lima* were variable in response to temperature and salinity changes. Population growth was reduced by a lower temperature for *Ostreopsis* sp. and *G. carpenteri*, by lower salinity, but resulted in higher nutritional values in relation to eicosapentaenoic acid and docosahexaenoic acid. Further studies are required on the physiological responses of dinoflagellates to non-favourable temperature and salinity conditions, including responses such as nutrient uptake rates, osmolyte production and essential fatty acid profiles. Such information will improve the understanding for successful establishment of benthic dinoflagellate assemblages, potential proliferation and likely uptake into marine food webs in southern temperate coastal habitats. The data presented in this study contribute baseline response information of tropical, toxic benthic dinoflagellates, critical for developing geographical range-expansion models for these species.

6.2 Introduction

Ciguatera is the most commonly reported seafood-induced illness worldwide (Arena et al., 2004; Tester et al., 2013) and is caused by consumption of fish that have accumulated ciguatoxins through their diet. Ciguatoxins are derived from dinoflagellates of the genus *Gambierdiscus*, which co-occur with the benthic dinoflagellates *Prorocentrum* and *Ostreopsis*, frequently present in high

abundances (Morton and Faust, 1997; Morton et al., 1992; Sparrow et al., 2017). As benthic dinoflagellate assemblages are usually found on macroalgal substrates, it has been hypothesised that ciguatoxins enter marine food webs mainly via consumption of algae by grazing fish (Randall, 1958). Consumption of these grazing fish by mesopredators, such as coral trout, barracuda and mackerel, result in accumulation of ciguatoxin higher in the food chain (Heimann et al., 2011; Lewis et al., 1991; Tester et al., 2013).

Occurrence of ciguatera in Australia has been associated with fish sourced from Queensland (Qld) coastal waters, including the Great Barrier Reef (GBR). In 2014 - 2016, however, five ciguatera outbreaks occurred south of Queensland, from locally caught Spanish mackerel within the South West Rocks coastal region (29 – 32 °S) in northern New South Wales (NSW) (Farrell et al., 2016a). The strengthened East Australian Current (EAC) during the Austral summer has, for more than 10 years, facilitated transfer of reef fish larvae from the southern GBR between January and May each year, into coastal marine habitats off the NSW coastline (Booth et al., 2007; Figueira and Booth, 2010). Whether Spanish mackerel caught in northern NSW consumed ciguatoxins derived from *Gambierdiscus* populations in local (i.e. NSW) marine food webs is unknown. Populations of *Gambierdiscus carpenteri*, *Prorocentrum* spp. and *Ostreopsis* sp. have, however, been recorded along the NSW coastline from Camden Haven River (32°S), in central NSW to Merimbula and Wonboyn River (37°S) in southern NSW (Ajani et al., 2013; Kohli et al., 2014). It is thought that the EAC also facilitated the southward geographic range expansion of benthic dinoflagellates from the GBR (Heimann et al., 2011; Kohli et al., 2014; Sparrow

and Heimann, 2016), as *Gambierdiscus* populations have been recorded seasonally at Merimbula during the Austral summer (Kohli et al., 2014), similar to records of the seasonal occurrence of reef fish (Booth et al., 2007). The tolerance of benthic dinoflagellates to a broad range of temperatures and salinities indicates an acclimation potential to temperate coastal environments (Kibler et al., 2012; Sparrow et al., 2017; Xu et al., 2016). The effect of environmental factors like temperature and salinity, however, on nutrient uptake and nutritional value, of benthic dinoflagellates is unknown.

Nutrients may be assimilated not only for growth, but also for physiological maintenance (such as cell growth, division, photosynthesis and metabolism). Tropical and temperate coastal marine habitats often experience periodic eutrophic conditions due to nutrient influx from anthropogenic and environmental disturbances (Ajani et al., 2011; Devlin and Brodie, 2005; Scanes et al., 2007; von Alvensleben et al., 2016). On the GBR, periodic pulses of water supply nutrients either by freshwater plumes in coastal habitats or by upwelling to mid-shelf and outer-shelf reefs, which occur infrequently during the Austral summer wet season (Andrews and Gentien, 1982; Devlin and Brodie, 2005). GBR waters are therefore, frequently oligotrophic but irregularly affected by an influx of nutrients (nitrogen from nitrate or ammonium and phosphorus from inorganic phosphate). Oligotrophic periods can cause a delay in the uptake of nitrate by some microalgae from one to 24 hours (Malerba et al., 2015), which could critically affect the assimilation of available nutrients by benthic dinoflagellates. Populations of tropical benthic dinoflagellates, transported southward by the EAC, may need to acclimate to higher ambient nutrient

concentrations and greater frequency of nutrient supply in more temperate waters.

The southward range expansion of *Gambierdiscus* into coastal NSW waters has the potential to increase the risk of ciguatera cases in temperate regions. In addition, as *Gambierdiscus* frequently co-occurs with toxin-producing *Prorocentrum lima* and *Ostreopsis* sp., which are associated with shellfish poisonings, responses of these associated dinoflagellates to lower temperatures and salinities should also be evaluated. Oyster farming operates along most of the NSW coastline and *P. lima* abundances are closely monitored as part of the NSW shellfish monitoring programs (Ajani et al., 2013). Despite this, the ecophysiological responses of *P. lima* to temperature and salinity variations remain poorly understood in Australia. As toxin transfer requires ingestion of the dinoflagellates, knowledge on the effects of temperature and salinity changes to their nutritional value is essential. Fatty acid profiles, an index of food quality, have been determined in one study on the benthic dinoflagellates, *Ostreopsis ovata*, *Coolia monotis* and *Amphidinium* sp. (Usup et al., 2008). Nutrient levels affect dinoflagellate population densities and are also essential for the production of osmolytes in response to lower salinity (von Alvensleben et al., 2016). Temperature, which alters membrane fluidity, and salinity, which potentially affects membranes through turgor changes, have been shown to affect fatty acid content and profiles in microalgae used as feed in aquaculture (Renaud and Parry, 1994; Renaud et al., 2002). Essential fatty acids in marine ecosystems are derived from primary producers, being pelagic and benthic microalgae. Dinoflagellates and diatoms are a major component of

primary producers, and thereby, are important sources of essential fatty acids in pelagic and benthic ecosystems (Carreón-Palau et al., 2013; Kelly and Scheibling, 2012; Litz et al., 2010; Wilson et al., 2001). The omega (ω)-3 essential LC-PUFAs, DHA and EPA and the ω-6 fatty acid, arachidonic acid (AA), are considered vital to human health (Huerlimann et al., 2014). Vertebrates must obtain these through diet, as they cannot be biosynthesized (Carreón-Palau et al., 2013; Fernandes et al., 2014; Li et al., 2012). High levels of EPA (20:5ω-3) and palmitoleic acid (16:1ω-7) are indicative of diatom origins. Dinoflagellates typically contain higher levels of DHA (22:6ω-3) and stearidonic acid (18:4ω-3) (Kelly and Scheibling, 2012; Li et al., 2012; Puccinelli et al., 2016); and presence of AA (20:4 ω-6) is often associated with macroalgae in benthic environments (Carreón-Palau et al., 2013; Wilson et al., 2001). The effect of temperature and salinity on fatty acid profiles (nutritional value) and nutrient uptake rates (osmolyte production) in benthic dinoflagellate assemblages are poorly investigated.

The purpose of this study was to determine the effect of lower temperature and salinity (such as might be associated with environmental disturbances on the GBR and/or transport southward by the EAC) on population growth, nutrient uptake rates and fatty acid profiles in the benthic dinoflagellates, *Gambierdiscus carpenteri, Prorocentrum lima* and *Ostreopsis* sp. isolated from the GBR. Such information is important in determining trophic impacts and the likely potential for southward geographical range expansion of *Gambierdiscus*, as well as the potential for bloom formation of benthic dinoflagellates.

6.3 Methods

6.3.1 Algal culture conditions

Gambierdiscus carpenteri (NQAIF 380), *Prorocentrum lima* (NQAIF 379) and *Ostreopsis* sp. (NQAIF 382) were isolated from water samples collected in the coral reef exhibit at the ReefHQ aquarium, Australia (Sparrow et al., 2017). Monocultures were established from the isolates and maintained (24 and 28°C with a 12:12 h photoperiod and 29 – 65 µmol m^2 s^{-1} irradiance) at the North Queensland Algal Identification/Culturing Facility (NQAIF) culture collection (James Cook University, Townsville, Australia) by the culture collection curator Stan Hudson. One-litre batch cultures were grown in 2 L Erlenmeyer flasks in K medium without sodium metasilicate nonahydrate and substituting sodium β-glycerophosphate with sodium dihydrogenphosphate monohydrate to an equivalent final concentration of phosphate (Keller et al., 1987). To generate sufficient biomass for fatty acid analysis, cultures were re-fertilized with nitrate (~55 mg L^{-1}) and phosphate (1.38 mg L^{-1}) when depleted during the 28-day experimental period.

Modified K medium (as above) was prepared at salinities of 36 and 26 in filtered GBR offshore seawater (fSW, Whatman GF/C 1.2 µm). Cultures were inoculated and sampled aseptically in a laminar flow (AES Environmental Pty Ltd fitted with a HEPA filter). For inoculation, mother cultures were concentrated by gravity filtration through a 20 µm mesh filter. Depending on species and growth behaviour, replicate cultures (n=3) were inoculated with cell densities between ~518 for *G. carpenteri*, ~2171 for *Ostreopsis* sp. and ~2988 cells. mL^{-1} for *P. lima*. Replicate mother cultures (1 L, n = 3) were maintained at 28 and 24

°C and acclimatized to salinities of 36 and 26 for three generations, over a period of three months. Mother cultures for each treatment were combined and concentrated, as outlined above, to the required biomass for inoculation of treatment and control cultures.

6.3.2 Estimation of culture cell concentrations and media nutrient concentrations

Cell densities were calculated at inoculation and harvest based on cell counts using a Sedgewick Rafter counting chamber on a Leica microscope at 400x magnification. Medium nitrate (NO_3^-), nitrite (NO_2^-) and phosphate (PO_4^{3-}) concentrations were determined every second day and following addition of NO_3^- and/or PO_4^{3-}, as described in von Alvensleben et al. (2013). Addition of nutrients depended on uptake for each culture and species.

Total nitrogen uptake (TN) was calculated as:

$$TN\left[mg.cell^{-1}\right]=t_x¿ \quad (1)$$

$$TN\ uptake\ rate\left[pg.cell^{-1}.day^{-1}\right]=\left(\frac{TN}{C_t}\times 1000\times 1000\times 1000\right)/t_{28} \quad (2)$$

Total phosphate uptake rate (TP) was calculated as:

$$TP\ uptake\ rate\left[pg.cell^{-1}.day^{-1}\right]=\left(\frac{t_x-t_y}{C_t}\times 1000\times 1000\times 1000\right)/t_{28} \quad (3)$$

Relative population growth (*r*) and cell division per day (*k*) were calculated following Wood et al. (2005a):

$$r = (\ln C_t - \ln C_0) / (t_{28} - t_0) \quad (4)$$

$$k = \frac{r}{\ln 2} \quad (5)$$

where, C_t is cell density at harvest; C_0 is cell density at inoculation; t_0 and t_{28} are initial and final culture time points (days); t_x and t_y are consecutive time points during the culture period (days).

6.3.3 Fatty acid analysis

Biomass samples for biochemical analyses were harvested from cultures by settling the entire biomass on ice. The biomass pellets were freeze-dried (Virtis benchtop 2K, VWR) and stored in air-tight vials under nitrogen at 4 °C until further analysis.

Fatty acids were extracted from freeze-dried samples in a single-step extraction and transesterification procedure adapted from Carvalho and Malcata (2005) and Cohen et al. (1988), as described in von Alvensleben et al. (2015), followed by GC-MS analysis. Briefly, 2 mL of freshly prepared methylation reagent (methanol:acetylchloride, 95:5 (v/v)) and 300 µL internal standard (nonadecanoic acid, (cat # 72332-1G-F), Sigma Aldrich, Australia), 0.2 mg L^{-1} in methanol) was added to approximately 5 mg (± 0.1 mg) dry biomass in Teflon-capped glass vials. Samples were heated at 100 °C for 1 h and allowed to cool, after which 1 mL of hexane was added. To ensure complete partitioning of the formed fatty acid methyl esters (FAMEs) into the hexane layer, samples were heated again at 100 °C for 1 min to form a single methanol/hexane phase. One mL of de-ionized water was then added to the cooled sample to facilitate phase

separation. The hexane phase containing the FAMEs was collected and filtered through a 0.2 µm PTFE syringe filter prior to injection on the GC column. All solvents were HPLC grade. Butylated-hydroxy-toluene (BHT) (0.01 %) was added as an antioxidant during the extraction.

Fatty acid analysis was carried out in scan-mode on an Agilent 7890 GC (DB-23 capillary column with a 0.15 µm cyanopropyl stationary phase, 60 m x 0.25 mm inner diameter) equipped with a flame ionisation detector (FID) and connected to an Agilent 5975C electron ionisation (EI) turbo mass spectrometer (Agilent Technologies, Australia). Fatty acid quantification was determined by comparison of peak areas with authentic external standards (Sigma Aldrich) and was corrected for recovery of internal standard (C19:0). Total fatty acid content was determined as the sum of all FAMEs.

6.3.4 Statistical analyses

Data were analysed via three-way ANOVAs, with α set to 0.05 to determine statistical significance (Statistica v13.2, Statsoft). Homogeneity of variances and normality were confirmed using the Levene's test and Q-Q plots, respectively. Missing data, due to sample pooling for *Ostreopsis* sp. (e.g. fatty acid profiling required large amounts of biomass (dry weight)) and loss of some replicates during lyophilisation for *G. carpenteri* and *P. lima*, limited factorial analysis of fatty acid profiles. Hence data on *Ostreopsis* sp. was not incorporated into statistical analyses. Also, some replicates were lost in the lyophilisation process of the biomass (*G. carpenteri* 28 °C, at a salinity of 26 and 24 °C for both salinities, and *P. lima* 28 °C and a salinity of 26, where only two of the three replicates remained). Thus, the effect of salinity on fatty acid profiles was

investigated for *P. lima* at 24 °C, the effect of temperature for *P. lima* at a salinity of 36 and the effect of species for *P. lima* and *G. carpenteri* at 28 °C and a salinity of 36, as these samples had three replicates.

To characterize species-specific responses to salinity and temperature, principal component analyses (PCAs) were conducted (Statistica v13.2, Statsoft). For the first PCA, analysis was based on population growth rates, total nitrogen and phosphate uptake rates and in the other PCA, sums of MUFA, PUFA, and SFA. Species were selected as the supplementary variable, temperature as an active variable and salinity as a grouping variable. Ward's hierarchical cluster analyses using squared Euclidean distances were conducted for objective description of physiological and biochemical clusters.

6.4 Results

6.4.1 Growth responses of benthic dinoflagellates to temperature and salinity changes

To investigate likely responses to exposure to inshore environmental conditions of *P. lima* (NQAIF379), *G. carpenteri* (NQAIF380) and *Ostreopsis* sp. (NQAIF 382), the effect of lower salinity and temperature was examined in a 28-day fully factorial time-course experiment conducted at 28 °C and salinities of 36 (control) and 26, and 24 °C for both salinities. Inoculation densities for the slow-growing *G. carpenteri* (0.5×10^6 cells L^{-1}) were limited by achievable population densities in mother cultures used for inoculation, which might be a consequence of larger cell size (3- or 4-fold) compared to *Ostreopsis* sp. and *P. lima*.

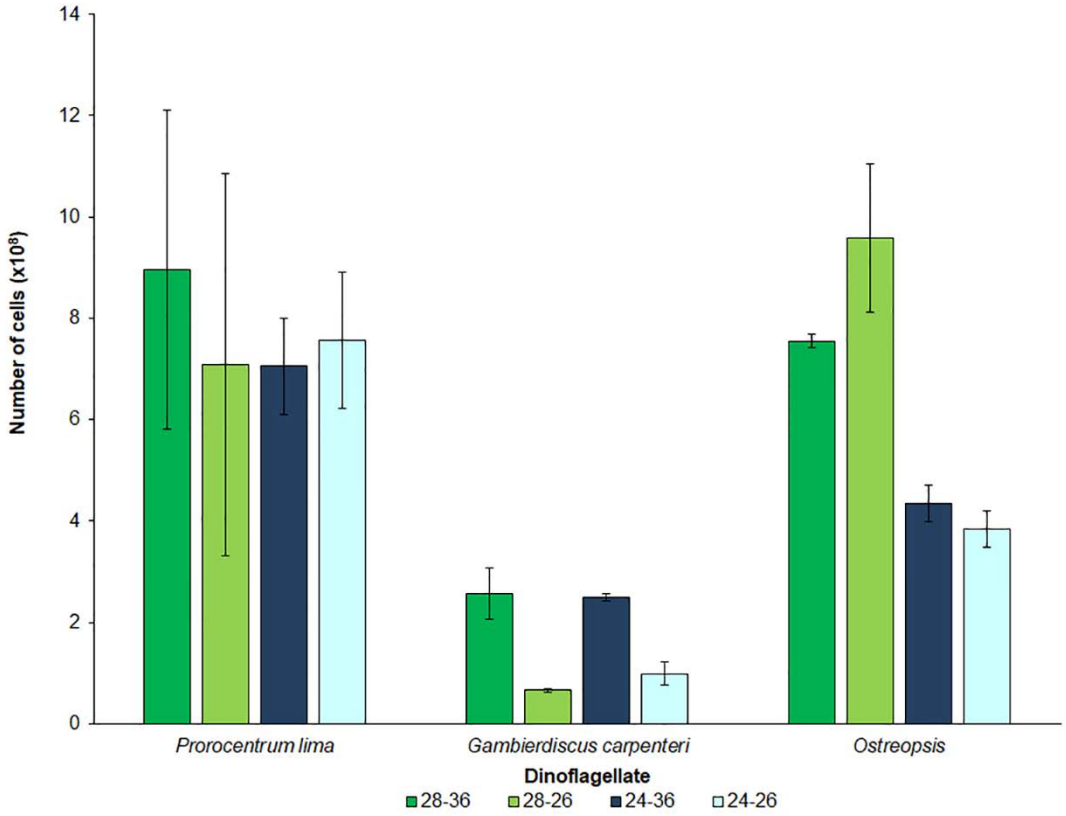

Figure 6.1 Final cell densities (cells L^{-1} on day 28) of benthic dinoflagellate cultures: NQAIF379, *Prorocentrum lima*; NQAIF380, *Gambierdiscus carpenteri*; NQAIF382, *Ostreopsis* sp. grown at 28°C and 24°C in salinities of 36 and 26. *n*=3. Standard error is shown.

For this reason, final population size achieved by *G. carpenteri* cannot be directly compared to those achieved by *P. lima* and *Ostreopsis* sp. (Fig. 6.1). As inoculation densities of *P. lima* and *Ostreopsis* sp. were comparable (2.9 and 2.2 x 10^6 cells L^{-1}), final population sizes achieved under the growth conditions can be compared. Final population sizes for *P. lima* and *Ostreopsis* sp. were very similar for 28 °C cultures at both salinities, but low temperature (24 °C) resulted in significantly lower population sizes of *Ostreopsis* sp., while this was not affected by salinity (Fig. 6.1). No temperature or salinity effect was observed for final population size of *P. lima* (Fig. 6.1). By contrast, final population size of *G. carpenteri* were negatively affected by the lower salinity, while temperature had no effect (Fig. 6.1).

A three-way ANOVA on the effect of temperature, salinity and species on population growth rates and doubling times indicated a significant effect of salinity, while temperature and species were not significant (Table 6.1). A significant interaction of temperature*species and salinity*species on population growth rate was also determined, while there was no significant interaction effect of temperature*salinity and temperature*salinity*species (Table 6.1). A Tukey's post hoc analysis showed a significant interaction effect of temperature with species (Table 6.1) for *G. carpenteri* with *P. lima* and *Ostreopsis* at a salinity of 26 (Supplementary Table S6.1, Appendix G). Salinity affected population growth rates of *G. carpenteri* significantly (Table 6.2) and a Tukey's post hoc test showed a significant interaction of salinity*species for *G. carpenteri* at a salinity of 36 with *P. lima* at 28 °C and *Ostreopsis* at 24 °C at a salinity of 26; and at 28 °C for *P. lima* at a salinity of 36 with *Ostreopsis* sp. and a salinity of 26 (Supplementary Table S6.1, Appendix G).

6.4.2 Nutrient uptake responses of benthic dinoflagellates to temperature and salinity changes

Total nitrogen (nitrite-corrected nitrate) uptake rates were steady (10-20 pg cell^{-1} day^{-1}) for *P. lima* irrespective of temperature and salinity up to day 20 when the medium was supplemented with nitrate (Figs 6.2 A and D). This resulted in a large increase in total nitrogen uptake rates for cultures at 28 °C for both salinities and 24 °C at a salinity of 26, whilst uptake rates remained steady for cultures at a salinity of 36 at this temperature. Further replenishment on day 22 for 28 °C cultures, where largest uptake rates were observed, maintained large total nitrogen uptake rates. In contrast, total nitrogen uptake rates fluctuated

Table 6.1 Summary statistics from three-way ANOVAs comparing population growth between benthic dinoflagellates: *Prorocentrum lima*, NQAIF379; *Gambierdiscus carpenteri*, NQAIF380; *Ostreopsis* sp. NQAIF382 at 28 and 24°C with salinities of 36 and 26 (n = 3 for each benthic dinoflagellate).

	Relative population growth (r)			Cell doubling time (k)	Total N uptake (pg cell^{-1} day^{-1})		Total phosphate (pg cell^{-1} day^{-1})	
	$F_{1,2}$	p	$F_{1,2}$	p	$F_{1,2}$	p	$F_{1,2}$	p
Temperature	0.11	0.75	0.01	0.92	0.22	0.65	**7.34**	**p<0.05**
Salinity	**10.81**	**p<0.05**	**9.89**	**p<0.05**	0.77	0.39	**5.80**	**p<0.05**
Species	0.43	0.65	1.07	0.36	**17.33**	**p<0.001**	**3.63**	**p<0.05**
Temperature*Salinity	0.11	0.75	0.95	0.34	0.10	0.75	1.87	0.18
Temperature*Species	**15.03**	**p<0.001**	**11.87**	**p<0.001**	**10.21**	**p<0.001**	**6.90**	**p<0.05**
Salinity*Species	**19.57**	**p<0.001**	**20.34**	**p<0.001**	**4.11**	**p<0.05**	1.14	0.34
Temperature*Salinity*Species	2.70	0.09	1.73	0.20	1.10	0.35	3.33	0.05

greatly for *G. carpenteri* which was amplified by low salinity at both temperatures (Figs 6.2B and E). At 28 °C, only small amounts of nitrate were secreted from cells into the culture medium on days 4, 6, 12 and 16 (Fig. 6.2B), but at a salinity of 26 large amounts of uptake oscillated with large amounts of secretion within the culture medium (Fig. 6.2E).

Table 6.2 Nutrient uptake rate (pg.cell^{-1}.day^{-1}) for total nitrogen (TN) and phosphate (PO_4^{3-}), relative population growth (*r*), and cell doubling time (*k*) over a 28-day period for benthic dinoflagellates NQAIF379, *Prorocentrum lima*; NQAIF380, *Gambierdiscus carpenteri*; NQAIF382, *Ostreopsis* sp. (n = 3 for each benthic dinoflagellate).

Temperature	28^{0}C		24^{0}C	
Salinity	36	26	36	26
NQAIF379 *Prorocentrum lima*				
r	0.19±0.01^{b}	0.18±0.02^{b}	0.20±0.00^{a,B}	0.21±0.01^{a,B}
k	0.28±0.02	0.26±0.03	0.29±0.01	0.30±0.01
TN	7.22±1.95^{a,B,2}	10.24±3.54^{a,B}	4.19±0.68^{b}	5.87±2.04^{a,Ø}
PO_4^{3-}	0.43±0.13^{A,b,Ø}	0.63±0.24^{A,b,2}	0.47±0.08^{A,b,Ø}	0.46±0.09^{A,b,Ø}
NQAIF380 *Gambierdiscus carpenteri*				
r	0.22±0.01$^{a,B,@}$	0.17±0.00^{b,A}	0.22±0.00$^{a,B,@}$	0.19±0.01^{b}
k	0.32±0.01	0.25±0.00	0.32±0.00	0.27±0.01
TN	4.16±0.86^{b}	2.36±0.80^{b}	3.70±0.09^{b}	1.41±0.57^{b,A}
PO_4^{3-}	0.38±0.08^{A,b,Ø}	0.31±0.04^{B,b}	0.35±0.06^{A,b,Ø}	0.50±0.12^{A,b,Ø}
NQAIF382 *Ostreopsis* sp.				
r	0.20±0.00^{a,B}	0.22±0.01^{a,B}	0.18±0.00^{b}	0.19±0.00^{b}
k	0.30±0.00	0.32±0.01	0.27±0.00	0.27±0.00
TN	1.00±0.13^{b,1}	0.82±0.11^{b,1}	2.95±0.61^{b}	5.91±0.26^{a,Ø}
PO_4^{3-}	0.15±0.01^{B,a,1}	0.18±0.02^{B,a}	0.33±0.06^{A,b,1}	0.73±0.05^{A,b,2}

$^{a, A}$ indicates driver of significant difference; ^{a} and $^{b, B}$ indicate significant differences.
$^{@}$ shares with *Ostreopsis* sp. 28^{0}C at a salinity of 26^{a} significant differences to *P. lima* 28^{0}C at a salinity of 26, *G. carpenteri* salinity of 26 at 28^{0}C and 24^{0}C, *Ostreopsis* sp. 24^{0}C at a salinity of 36 and 26.
^{Ø} indicates not significantly different to any treatments.

Ostreopsis sp., on the other hand, showed steady uptake rates of total nitrogen, similar to *P. lima* at salinities of 36 and 26 at 24 °C (Figs. 6.2 C and F). By contrast to *P. lima*, however total nitrogen uptake rates were generally lower at 28 °C for both salinities.

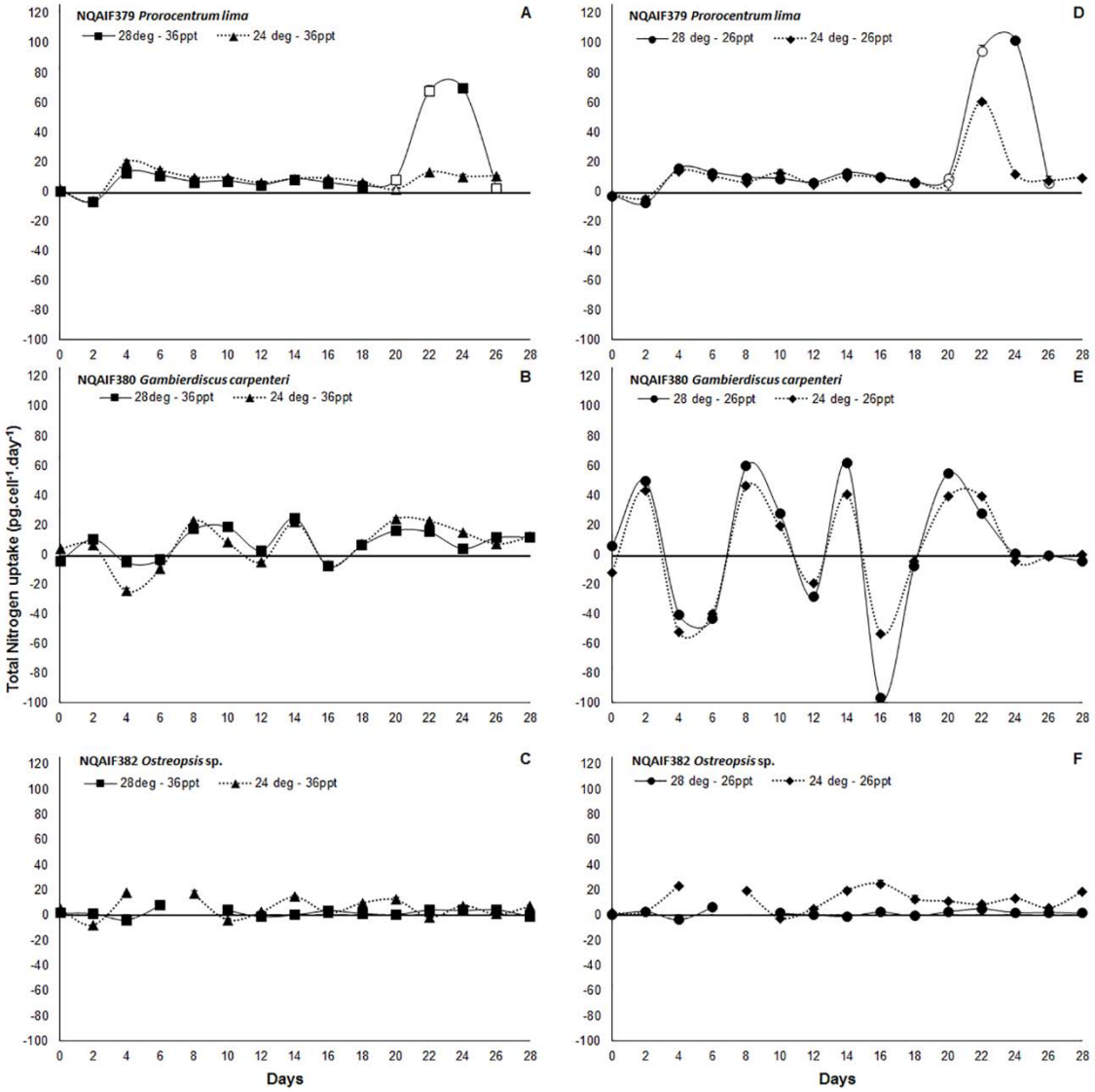

Figure 6.2 Total nitrogen uptake (pg.cell^{-1}.day^{-1}) over 28 days at 28°C and 24°C for benthic dinoflagellates: *Prorocentrum lima* at a salinity of 36 (A) and 26 (D); *Gambierdiscus carpenteri* in a salinity of 36 (B) and 26 (E); *Ostreopsis* sp. at a salinity of 36 (C) and 26 (F). *n*=3. Standard error is visible when larger than data points.

A three-way ANOVA indicated a significant effect of species and a significant interaction effect of temperature*species and salinity*species on total nitrogen uptake rates, while temperature and salinity as well as the interaction of temperature*salinity and temperature*salinity*species were not significant (Table 6.1). *P. lima* had the highest nitrogen uptake rates of 10.24 and 7.22 pg cell^{-1} day^{-1} at 28 °C and at 24 °C, with the latter only exceeded by *Ostreopsis* sp. at 24 °C and salinity 26 (Table 6.2). A Tukey's post hoc analysis showed that

the significance of the effect of species and the interaction of temperature*species and salinity*species was driven by the large total nitrogen uptake rate of *P. lima* at 28 °C and a salinity of 26 (Table 6.2, Supplementary Table S6.2, Appendix H), which required refertilisation with nitrogen twice over the cultivation period (Figs 6.2A and D).

Total phosphate uptake was not influenced by temperature or salinity for *P. lima*, requiring replenishment of the medium with phosphate every 2-days (Fig. 6.3 A and D). Similar to growth performance and total nitrogen uptake patterns, total phosphate uptake by *G. carpenteri* fluctuated greatly at a salinity of 26 for both temperatures, with large uptake observed between inoculation and day 2 (Fig. 6.3 E). Following replenishment of the medium on day 14, however, uptake rates were three times greater for cultures under 24 °C and a salinity of 26 compared to cultures at 28 °C and that salinity (Fig. 6.3 E). By contrast, and irrespective of phosphate replenishment of the medium, total phosphate uptake rates did not differ greatly for cultures at a salinity of 36 (Fig. 6.3 B), which was on average similar to rates observed for *P. lima* (Figs. 6.3 A and D). For *Ostreopsis* sp., large phosphate uptake rates were observed at 24 °C and a salinity of 26, requiring frequent replenishment of the medium (Fig. 6.3 F), while uptake rates at 28 °C and a salinity of 26 were comparable to those at 28 °C for a salinity of 36 (Figs. 6.3 C and F) and not enhanced by phosphate addition to the medium (Fig. 6.3 F). Phosphate uptake rates were initially higher at 28 °C at a salinity of 36 up to day 10 compared to cultures at 24 °C, and luxury phosphate uptake was observed only for cultures at 24 °C at a salinity of 36 on day 8 (Fig. 6.3 C).

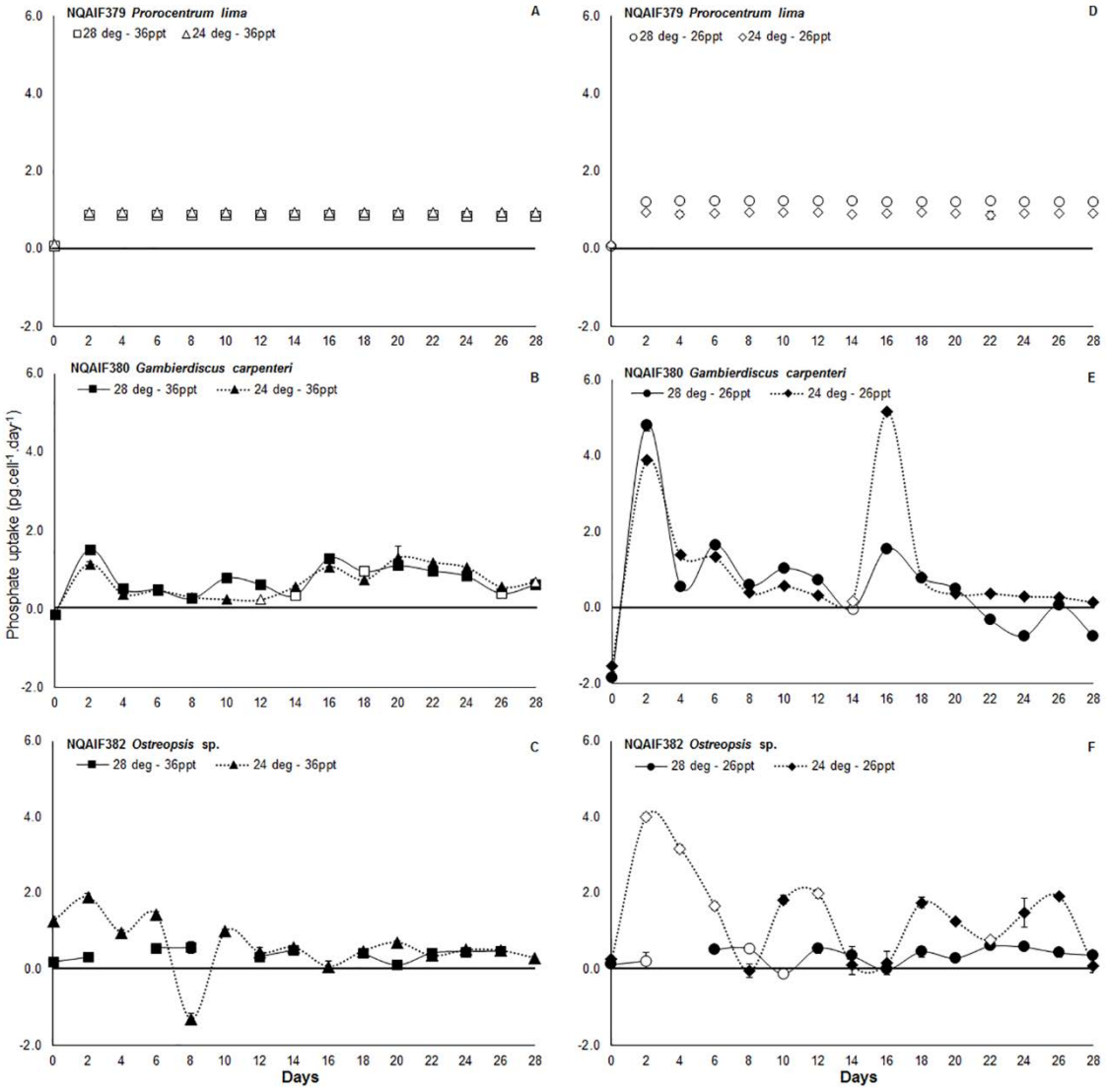

Figure 6.3 Phosphate uptake (pg.cell^{-1}.day^{-1}) over 28 days at 28°C and 24°C for benthic dinoflagellates: *Prorocentrum lima* in a salinity of 36 (A) and 26 (D); *Gambierdiscus carpenteri* in a salinity of 36 (B) and 26 (E); *Ostreopsis* sp. in a salinity of 36 (C) and 26 (F). *n*=3. Standard error is shown.

A three-way ANOVA indicated a significant effect of temperature, salinity, species and temperature*species interaction on total phosphate uptake rates, while temperature*salinity, salinity*species and temperature*salinity*species interactions were not significant (Table 6.1). Highest phosphate uptake rates of 0.73, 0.63 and 0.5 pg cell^{-1} day^{-1} were observed at a salinity of 26 at 24 °C for *Ostreopsis* sp., at 28 °C for *P. lima* and 24 °C for *G. carpenteri*, respectively, while lowest uptake rates were observed for *Ostreopsis* sp. at 28 °C for both

salinities (Table 6.2). A Tukey's post hoc analysis showed that the significance of species and temperature and temperature*species interaction was driven by very low total phosphate uptake rates of *Ostreopsis* sp. at 28 °C for both salinities (Table 6.2). The effect of salinity was driven by a significant difference of *Ostreopsis* sp. at 28 °C and a salinity of 36 to phosphate uptake rates at 24 °C salinity 26 and *P. lima* at 28 °C and salinity 26 (Table 6.2, Supplementary Table S6.3, Appendix H).

A principal component analysis (PCA) showed species-specific trends of population growth rates and total nitrogen uptake rates, with the first two components of the PCA explaining 81.92 and 16.87% of the variability (Fig. 6.4). PC1 separated species by population growth rate, while PC2 separated species by total nitrogen uptake rate. For *G. carpenteri* higher total nitrogen uptake rates were associated with higher growth rates, irrespective of temperature, while for *Ostreopsis* highest growth rate occurred at the lowest nitrogen uptake rates, irrespective of salinity, but it separated out at the low salinities according to temperature preference. By contrast, *P. lima* was positively correlated with PC2, while the distribution along PC1 was heterogeneous both with regards to temperature and salinity. A cluster analysis confirmed species-specific grouping with temperature, salinity and total nutrient uptake rates with growth rates (Supplementary Fig. S6.1, Appendix I).

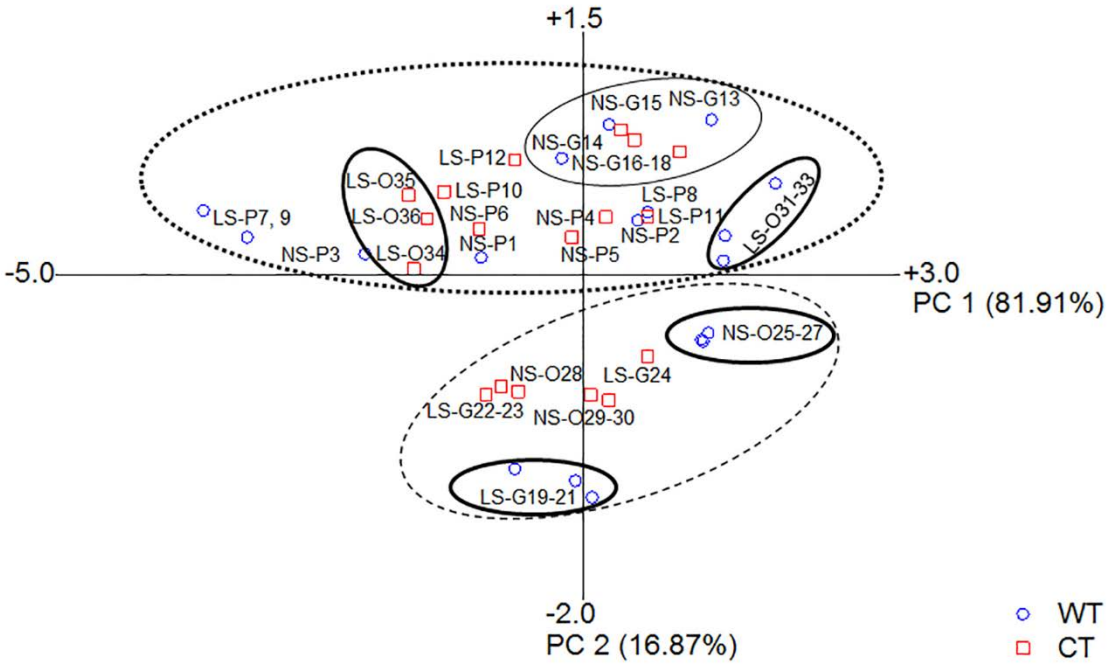

Figure 6.4 A principal component analysis showing the relationship between growth rates and nutrient uptake rates of benthic dinoflagellates: G *Gambierdiscus carpenteri*; P *Prorocentrum lima*; O *Ostreopsis* sp.; *LS* low salinity, 26; *NS* normal salinity, 36; *WT* warm temperature, 28 °C; *CT* cool temperature, 24 °C.

6.4.3 Effect of salinity and temperature on fatty acid profiles of benthic dinoflagellates

Irrespective of species, temperature or salinity, palmitic acid (C16:0) was present at highest concentrations (≤ 2 to ≥ 8 mg g^{-1} DW) and at a 30-50% contribution to the FA profile, followed by eicosapentaenoic acid (EPA; C20:5) and docosahexaenoic acid (DHA; C22:6) and oleic acid (C18:1), whilst arachidonic acid (C20:4) was not detected in any of the species under any cultivation condition (Table 6.3, Figure 6.5). Palmitic acid contributed 40-50% to the total fatty acid content in *G. carpenteri*, whilst it was between 30-35% for *P. lima* and *Ostreopsis* sp. (Figure 6.5). Percent oleic acid concentrations were also lower in *P. lima* and *Ostreopsis* sp. (~5-10%), with no large effects of salinity or temperature observed, whilst percent contribution to fatty acids were

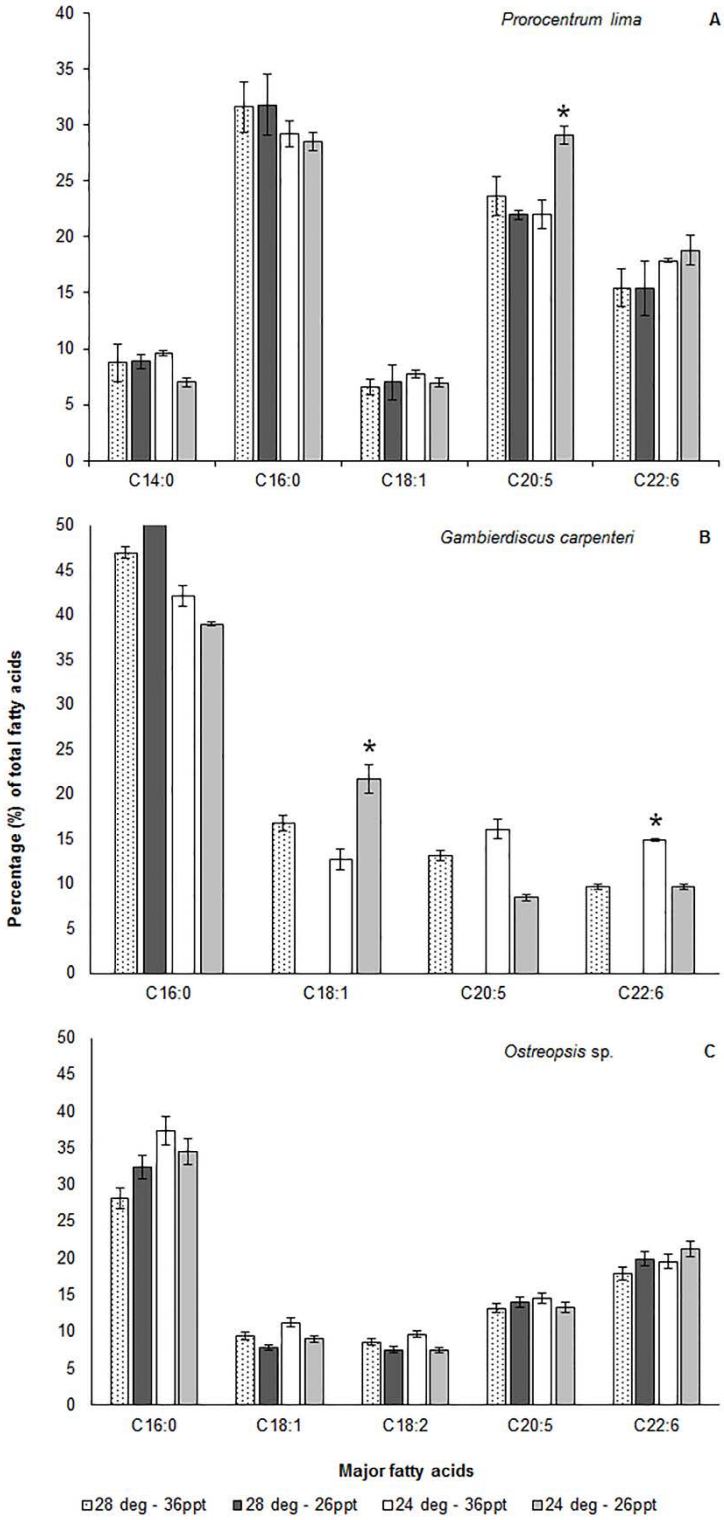

Figure 6.5 The effect of temperature and salinity on percentage of major fatty acids, palmitic acid (C16:0); oleic acid (C18:1ω-9); eicosapentaenoic acid (EPA C20:5ω-3); and docosahexaenoic acid (DHA C22:6ω-3) as % of total FAME for benthic dinoflagellates (A) *Prorocentrum lima* (NQAIF379); (B) *Gambierdiscus carpenteri* (NQAIF380); (C) *Ostreopsis* sp. (NQAIF382). *n*=3., except *Ostreopsis* sp., samples were combined. Standard error is shown.

(13-22%) for *G. carpenteri*, with the highest relative contribution observed at 24 °C and a salinity of 26. Percent EPA was similar in *Ostreopsis* sp. compared to *G. carpenteri*, but in *G. carpenteri* amounts were two-fold lower at a salinity of 26 at 24 °C, compared to 36 for both temperatures, whilst such effects were not observed in *Ostreopsis* sp. Highest relative amounts of EPA were observed for *P. lima* (~25-30%) with highest levels at 24 °C at a salinity of 26. Whilst relative DHA levels were comparable between *P. lima* and *Ostreopsis* sp. (~20-25%), DHA levels were lower than EPA levels for *P. lima* and slightly higher for *Ostreopsis* sp. No treatment effect on DHA levels was observed for these two species, but appeared to be slightly higher at 24 °C for *P. lima*. DHA levels were lowest in *G. carpenteri* (~10-15%), with highest levels observed at 24 °C and a salinity of 36.

In general, total fatty acid content was lowest for *Ostreopsis* sp. at a salinity of 36 for 24 and 28 °C, whilst highest total fatty acid contents were observed for *G. carpenteri* at a salinity of 36 for both temperatures (Table 6.3). *Ostreopsis* sp. also had the lowest content of polyunsaturated fatty acids (PUFA) at 28 °C for both salinities and at 24 °C at a salinity of 36 along with *G. carpenteri* at a salinity of 26 (Table 6.3). Generally, fatty acid profiles were dominated by SFA and PUFA at similar levels with much lower levels of mono-unsaturated fatty acids (MUFA) being observed (Table 6.3). Similarly, ω-3 fatty acids were present at all temperatures and salinities for all species and content was at least three times greater than ω-6 fatty acids, the latter were not detected in *P. lima* at 24 °C at either salinity (Table 6.3). Due to high amounts of EPA

Table 6.3 Fatty acid composition in dry weight (mg. g^{-1} dry weight) of benthic dinoflagellates: NQAIF379, *Prorocentrum lima*; NQAIF380, *Gambierdiscus carpenteri*; NQAIF382, *Ostreopsis* sp. grown at 28°C and 24°C and at salinities 36 and 26.

		Prorocentrum lima NQAIF379				*Gambierdiscus carpenteri* NQAIF380				*Ostreopsis* sp. NQAIF382			
		28°C		24°C		28°C		24°C		28°C		24°C	
Fatty acid	Common Name	36[B, C]	26	36[A, B]	26[A]	36[C]	36	26	36	26	36	26	26
C14:0	Myristic acid	1.27±0.48	1.47±0.86	1.00±0.12	1.05±0.05	0.70±0.02	0.54±0.02	0.60±0.03	0.51	0.62	-	0.74	
C16:0	Palmitic acid	**4.56±1.50**	5.29±3.15	**3.03±0.45**	**4.28±0.30**	**8.08±1.16**	6.91±0.28	4.57±0.65	1.94	3.06	2.49	4.61	
C16:1	Palmitoleic acid	**0.71±0.04**	0.75±0.12	**0.60±0.02**	**0.67±0.00**	-	0.53±0.00	0.60±0.06	0.51	0.51	0.52	0.57	
C18:0	Stearic acid	0.90±0.24	0.97±0.40	0.80±0.02	0.77±0.02	0.69±0.04	0.49±0.02	0.60±0.03	0.55	0.59	-	0.64	
C18:1 ω-9	Oleic acid	**0.92±0.16**	1.07±0.35	**0.80±0.05**	**1.04±0.02**	**2.89±0.50**	2.09±0.33	2.56±0.52	0.65	0.74	0.75	1.21	
C18:2 ω-6	Linoleic acid	**0.34±0.30**	0.51±0.00	-	-	**0.91±0.07**	0.76±0.03	0.66±0.03	0.59	0.71	0.64	1.00	
C20:4 ω-6	Arachidonic acid (**AA**)	-	-	-	-	-	-	-	-	-	-	-	
C20:5 ω-3	**EPA**	3.33±0.71	3.59±1.93	**2.29±0.33**	**4.36±0.41**	2.26±0.32	2.65±0.36	0.99±0.12	0.91	1.33	0.97	1.77	
C22:6 ω-3	**DHA**	2.25±0.85	2.61±1.70	**1.85±0.20**	**2.83±0.40**	1.67±0.19	2.45±0.14	1.13±0.18	1.23	1.88	1.30	2.83	
	TFA	14.27±3.94	16.26±8.51	**10.37±1.16**	**15.00±1.15**	17.21±2.25	16.42±1.12	11.72±1.59	6.88	9.45	6.67	13.36	
	ΣSFA	6.73±2.20	7.73±4.41	**4.83±0.58**	**6.09±0.36**	9.48±1.20	7.95±0.25	5.78±0.70	3.00	4.28	2.49	5.99	
	ΣMUFA	**1.63±0.20**	1.83±0.47	**1.40±0.07**	**1.71±0.03**	**2.89±0.50**	2.62±0.33	3.16±0.58	1.16	1.25	1.27	1.77	
	ΣPUFA	5.91±1.63	6.71±3.63	**4.14±0.53**	**7.19±0.81**	4.84±0.58	5.86±0.54	2.78±0.33	2.72	3.92	2.91	5.60	
	Σ ω-3	5.57±1.57	6.20±3.63	4.14±0.53	**7.19±0.81**	3.93±0.51	5.10±0.51	2.12±0.30	2.14	3.21	2.27	4.60	
	Σ ω-6	**0.34±0.30**	0.51±0.00	-	-	**0.91±0.07**	0.76±0.03	0.66±0.03	0.59	0.71	0.64	1.00	
	ω-6/ω-3	0.06±0.06	0.10±0.06	-	-	0.23±0.02	0.15±0.01	0.31±0.03	0.28	0.22	0.28	0.22	

EPA: Eicosapentaenoic acid; DHA: Docosahexaenoic acid. One-way ANOVA statistical analysis conducted for treatments with n=3: [A]*Prorocentrum lima* between salinities at 24°C; [B]*P. lima* between temperatures at a salinity of 36; [C]between *P. lima* and *Gambierdiscus carpenteri* at 28°C and a salinity of 36. Significant differences indicated in bold and correspond to summary statistics in Table 4.

(C20:5; generally, between 2 to < 5 mg g^{-1} dry weight (DW)), except for *Ostreopsis* sp.) and DHA (C22:6; generally, > 1 and < 3 mg g^{-1} DW) and low amounts of linoleic acid (LA; C18:2, 0 to ≤ 1 mg g^{-1} DW), ω6/ω-3 ratios were low, ranging from very low (0 to ≤ 0.1 ± 0.66) for *P. lima* at all salinities and temperatures to moderately low (0.15 ± 0.01 to 0.31 ± 0.03) for the other species (Table 6.3).

A one-way ANOVA investigating the effect of salinity on fatty acid profiles in *P. lima* at 24 °C showed a significant effect on all FA levels (Table 6.4), except for C14:0, which were generally higher at the lower salinity (Table 6.3). By contrast, although FA levels were generally lower at 24 °C compared to 28 °C for *P. lima* (Table 6.3), a one-way ANOVA on the effect of temperature at a salinity of 36 showed only a significant effect on levels of palmitoleic acid (C16:1) (Table 6.4). A significant effect of species was detected for C16:0, C18:1, C18:2 for *P. lima* and *G. carpenteri* at 28 °C and a salinity of 36 (Table 6.4), due to the 2-3 times higher concentration of these fatty acids in *G. carpenteri*, with concentration differences in C18:1 and C18:2 driving the significance of sum of MUFA and ω-6 fatty acids, respectively (Table 6.3).

A PCA showed clear separation of species based on sum of SFA and PUFA, with the first two components of the PCA explaining 75.41 and 24.09% of the variability (Fig. 6.6). PC1 separated species by ΣSFA, while PC2 separated species by ΣPUFA. For *G. carpenteri* high ΣSFA were associated with medium to low levels of ΣPUFA, with lowest ΣPUFA observed for 24 °C and a salinity of 26. In contrast, *Ostreopsis* was characterized by low ΣSFA content, irrespective

of salinity, but ΣPUFA were positively associated with low salinity. The PCA plot shows partial separation of *P. lima* along PC1 with salinity, while the distribution along PC2 was heterogeneous both with regards to temperature and salinity. A cluster analysis confirmed a grouping of ΣSFA and ΣPUFA with ΣMUFA grouped with nutrient uptake rates and growth rates (Supplementary Figure S6.1, Appendix I).

Table 6.4 Summary statistics from one-way ANOVAs comparing fatty acid contents (mg. g^{-1} dry weight) for NQAIF379, *Prorocentrum lima* between: [A]salinities 36 and 26 at 24°C; [B]temperatures 28°C and 24°C at a salinity of 36; and with [C]NQAIF380, *Gambierdiscus carpenteri* at 28°C and a salinity of 36.

	NQAIF 379				NQAIF 379 & 380	
	Salinity[A]		Temperature[B]		Dinoflagellate	
	$F_{1,4}$	P	$F_{1,4}$	P	$F_{1,4}$	P
C16:0	16.03	p<0.05			*10.44*	*p<0.04*
C16:1	38.21	p<0.004	18.69	p<0.02		
C18:1 n-9	52.72	p<0.002			*43.05*	*p<0.003*
C18:2 n-6					*10.4*	*p<0.04*
C20:5n-3	46.04	p<0.003				
C22:6n-3	13.95	p<0.05				
TFA	24.02	p<0.01				
ΣSFA	10.26	p<0.05				
ΣMUFA	50.28	p<0.003			*16.93*	*p<0.02*
ΣPUFA	29.94	p<0.006				
Σ ω-6					*20.67*	*p<0.02*

A PCA showed clear separation of species based on sum of SFA and PUFA, with the first two components of the PCA explaining 75.41 and 24.09% of the variability (Fig. 6.6). PC1 separated species by ΣSFA, while PC2 separated species by ΣPUFA. For *G. carpenteri* high ΣSFA were associated with medium to low levels of ΣPUFA, with lowest ΣPUFA observed for 24 °C and a salinity of 26. By contrast, *Ostreopsis* was characterized by low ΣSFA content,

irrespective of salinity, but ΣPUFA were positively associated with low salinity. The PCA plot shows partial separation of *P. lima* along PC1 with salinity, while the distribution along PC2 was heterogeneous both with regards to temperature and salinity. A cluster analysis confirmed a grouping of ΣSFA and ΣPUFA with ΣMUFA grouped with nutrient uptake rates and growth rates (Supplementary Figures S6.1, Appendix F and S6.2, Appendix G).

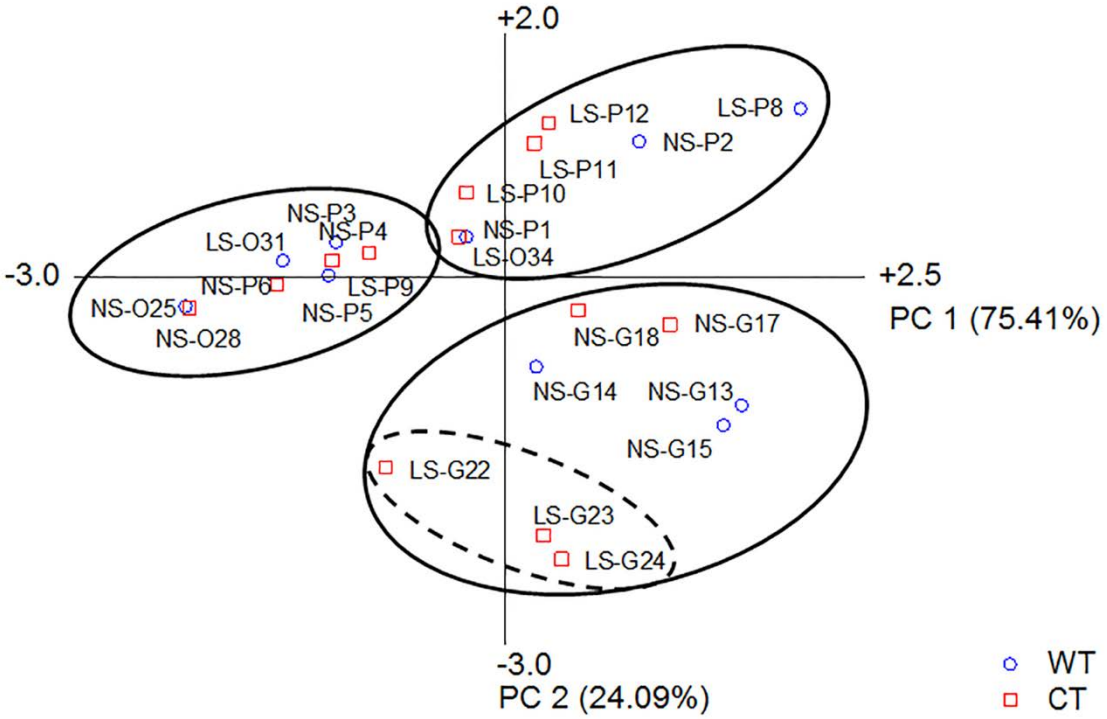

Figure 6.6 A principal component analysis showing the relationship between the sum of saturated, monounsaturated and polyunsaturated fatty acids for benthic dinoflagellates: G *Gambierdiscus carpenteri*; P *Prorocentrum lima*; O *Ostreopsis* sp.; *LS* low salinity, 26; *NS* normal salinity, 36; *WT* warm temperature, 28 °C; *CT* cool temperature, 24 °C.

6.5 Discussion

Temperature and salinity changes, as well as nutrient supplies, affect population growth rates and essential ω-3 LC-PUFA contents of microalgae, which as primary producers, form the base of the food web. Thus, potentially, the broad tolerance range to temperature and salinity of the benthic toxic dinoflagellates *G. carpenteri, P. lima* and *Ostreopsis* sp., which often occur

naturally in an assemblage complex (Sparrow et al., 2017), could facilitate population expansions into new geographic locations (Granéli et al., 2011; Sparrow et al., 2017; Xu et al., 2016). Population sizes, growth rates and nutritional profiles of these organisms from the GBR determine the potential for toxin transfer via the food web. In order to develop range expansion models for potentially toxic benthic dinoflagellates from tropical to temperate regions, it is critical to determine the effects of lower temperature and salinity on population growth rates, nutrient utilization and ω-3 LC-PUFA contents. Such effects have previously not been investigated. Active growth has been recorded for *Gambierdiscus, Prorocentrum* and *Ostreopsis* between 15 and 33 °C and salinities of 15 to 40 (Ben-Gharbia et al., 2016; Gillespie et al., 1985; Kibler et al., 2012; Morton et al., 1992). Maximum growth rates of populations for *P. lima* and *Ostreopsis* at 27 °C were observed to peak at salinities of 30 and 33, respectively (Morton and Norris, 1990; Morton et al., 1992). By contrast, optimal growth rates for *Gambierdiscus* species at 26 °C varied between salinities of 25 and 35. Growth rates of populations peaked at a salinity of 27 for *G. carpenteri,* isolated from Guam in the Pacific Ocean (Kibler et al., 2012). Although studies of growth response to salinity were conducted at similar temperatures to this study (28 and 24 °C), population growth responses differed. In this study, population growth for *P. lima* and *Ostreopsis* sp. was comparable at salinities of 36 and 26, but a salinity of 26 population growth rate of *G. carpenteri* was reduced significantly. While salinity tolerance range can indicate the likely ability to acclimatise in different coastal marine habitats, including estuaries, tidal rocky shores and riverine environments, geographic origin was shown to influence the upper and lower thermal limits for *Gambierdiscus* and *Ostreopsis*

(Granéli et al., 2011; Kibler et al., 2012; Xu et al., 2016). Recently, two strains of *G. carpenteri* isolated from the same latitude, but from different habitats, showed different thermal and salinity responses in terms of growth rates, suggesting that acclimation to local environmental conditions may result in differences within species (Sparrow et al., 2017). Similarly, *Ostreopsis ovata* in the Mediterranean appears to have acclimated to local environmental conditions, as population growth was observed to increase as temperature increased from 16 – 30 °C in the Tyrrhenian Sea, but reached a maximum at 16 °C in the North Adriatic Sea (Granéli et al., 2011). In this study, a tropical *Ostreopsis* sp. had higher population growth rate at 28 °C than at 24 °C, which was similar to observations by Granéli et al. (2011) in the Tyrrhenian Sea, but differed from other studies in tropical regions, which recorded peak growth at 25 °C for *Ostreopsis siamensis*, and *O. heptagona* (Morton and Norris, 1990; Morton et al., 1992). Population growth for *Prorocentrum* species, including *P. lima*, peaked at mid-tolerance range temperatures of 26 – 27 °C (Morton and Norris, 1990; Morton et al., 1992), but maximum growth for *Gambierdiscus* varied between 26 – 31 °C depending on species (Bomber et al., 1988a; Kibler et al., 2012). In comparison, population growth for *P. lima* and *G. carpenteri* in this study was not affected by temperature. Adaptability or acclimatization capacity to local environmental conditions has been the suggested rationale for inconsistencies between geographic locations for *G. carpenteri* and *O. ovata* species in response to temperature and salinity (Granéli et al., 2011; Sparrow et al., 2017; Xu et al., 2016). Intensive sampling and phylogenetic analysis of *Ostreopsis* in Japan identified an *O.* cf *ovata* complex, where strain distribution appeared to infer geographic preferences (Sato et al., 2011). Sporadic benthic

dinoflagellate surveys have recorded the presence of *Gambierdiscus* spp. along the Qld coast and seasonally at Merimbula on the southern NSW coast. Whether populations in Merimbula are derived through range expansion from tropical locations to the north like reef fish, whose thermal survival threshold is similar, about 17 °C (Figueira and Booth, 2010; Kohli et al., 2014), and have acclimated to temperate environmental conditions remains to be established.

Benthic dinoflagellate species, *G. carpenteri*, *P. lima* and *Ostreopsis* sp. have been recorded within temperate estuarine and riverine environments (Ajani et al., 2013; Kohli et al., 2014), where salinity and temperature changes would make establishment of tropical dinoflagellate populations difficult. Similar to mixed benthic dinoflagellate assemblages in tropical regions, at Merimbula, *G. carpenteri* co-occurred with other benthic dinoflagellates, including *P. lima* and *Ostreopsis* sp. (Kohli et al., 2014). During periods of lower salinities, survival of *G. carpenteri* in coastal habitats on the GBR was observed to improve within mixed benthic dinoflagellate assemblages (Sparrow et al., 2017). To truly understand the potential for *G. carpenteri* to survive in colder marine habitats, such as Merimbula, NSW, research on population growth responses at cooler temperatures in different salinities needs to be conducted on single species as well as with mixed benthic dinoflagellate assemblages.

Oligotrophic waters of the GBR have low average nitrogen and phosphorus concentrations of 0.0014 and 0.015 µg. L^{-1}, respectively (Furnas et al., 2005). Nutrients are supplied periodically by freshwater flood plumes, sediment resuspension or upwelling (Andrews and Gentien, 1982). In comparison,

temperate cooler waters are nutrient-rich, with average concentrations of nitrogen and phosphorus ranging from 32 and 9.4 µg. L^{-1} in central NSW to 0.8 and 0.1 µg. L^{-1} at Merimbula in southern NSW, respectively (Scanes et al., 2007). Since nutrient availability and uptake rates determine population growth rates, nutrient uptake rates (pg.$cell^{-1}$.day^{-1}) were used here to compare the results with those published. For the GBR isolates, based on highest and lowest nitrogen uptake rates observed, population densities of 329 – 972, 134 – 327, and 232 – 1,671 cells L^{-1} of *G. carpenteri*, *P. lima* and *Ostreopsis* sp. could be supported, respectively, whilst reported average phosphorus concentrations would sustain larger population densities of 7,945 -12,815, 6,306 -9,239, and 5,442 – 26,484, respectively. This confirms reports that phytoplankton populations are nitrogen-limited on the GBR (Wooldridge et al., 2015). Based on documented nutrient loads (Supplementary Table S6.4, Appendix J) and calculated nutrient consumption rates, GBR nutrient concentrations would maintain the smallest populations of these benthic dinoflagellates, while populations of *P. lima* and *O. ovata* would reach millions of cells per litre in nutrient-rich Mediterranean waters and one order of magnitude larger populations of *Gambierdiscus toxicus* would be sustained by average nutrient concentrations in the Caribbean (Supplementary Table S6.4, Appendix J). Calculated nitrogen and phosphorus uptake rates for the Caribbean *G. toxicus* and the Mediterranean *P. lima* and *O. ovata* were 5 – 100 times higher (Supplementary Table S6.5, Appendix K) than uptake rates observed in this study, suggesting that uptake rates may be linked to the nutrient history that the dinoflagellates experience. In addition, while growth rates appear to be nitrogen-limited on the GBR and in the Caribbean, population size appears to

be phosphorus-limited in the Mediterranean (Supplementary Table S6.5, Appendix K).

Nutrient-rich waters in colder temperate regions are likely to exceed growth requirements for maintaining population sizes of toxic dinoflagellates, potentially supporting bloom development as observed with *O. ovata* in the Mediterranean (Accoroni et al., 2011) and high seasonal abundances of *G. carpenteri* in Merimbula, NSW (Kohli et al., 2014). To date there is an absence of research on whether unfavourable conditions for benthic dinoflagellate cultures affect nutrient uptake rates and growth rates. In this study, nutrient uptake rates were influenced significantly by species, which were driven by the respective temperature and salinity preferences of the species. Nitrogen utilization by *G. carpenteri* was linked to growth rates, while stress-induced higher nitrogen uptake rates by *P. lima* and *Ostreopsis* sp. was not translated into growth, as growth rates were low. Benthic dinoflagellates may show low population growth rates in response to changes in temperature and salinity (Sparrow et al., 2017). Under temperature and salinity stress conditions it is likely that costly metabolic energy would be diverted from growth to physiological responses for survival, such as the production of osmolytes and heat-shock proteins (Kibler et al., 2012; Rosic et al., 2011; von Alvensleben et al., 2016). Higher nitrogen uptake rates that were not translated into growth, may have been utilized for N-based osmolytes, such as proline, taurine and glycine betaine, which have been frequently found in microalgae (Keller et al., 1999; Tevatia et al., 2015; von Alvensleben et al., 2016). Dimethylsulfoniopropionate (DMSP) is a suggested osmolyte in dinoflagellates, including several species of *Prorocentrum* (Caruana

and Malin, 2014), however, the formation of DMSP remains to be established for *P. lima*, *G. carpenteri* and *Ostreopsis*. Synthesis of DMSP commences with ATP-assisted sulphur assimilation that leads to the formation of the sulphated amino acid methionine, which is followed by oxidative decarboxylation to DMSP (Gage et al., 1997; Stefels, 2000). While osmolyte production was not investigated here, the highest uptake rates for nitrogen and phosphate were observed at lower population growth rates at a salinity of 26 for *P. lima* at 28 °C and *Ostreopsis* sp. at 24 °C, suggesting potential production of DMSP as an osmolyte in response to environmental stress. By contrast, population growth for *G. carpenteri* declined with an equivalent reduction in nutrient uptake under unfavourable conditions. Research on response of benthic dinoflagellates to environmental stressors, including potential production of osmolytes, would provide a clearer understanding of the adaptability to dynamic marine coastal habitats and southward range expansion of benthic dinoflagellates from the GBR into colder marine habitats.

Other than abundance and population growth rates, nutritional profile of benthic dinoflagellates is important for bioaccumulation of ciguatoxins into marine food webs. It is generally accepted that ω-3 fatty acids (EPA and DHA), stearidonic acid (C18:4 ω-3) and octadecapentaenoic acid (C18:5 ω-3) are characteristic of dinoflagellates, however, most research has been conducted on planktonic dinoflagellates (Litz et al., 2010; Mansour et al., 1999; Usup et al., 2008), with a paucity of research on the fatty acid profiles of benthic dinoflagellates. An analysis of fatty acids for planktonic and benthic dinoflagellates, including *Ostreopsis*, isolated from Malaysian waters did not detect the characteristic 18:4

ω-3 and 18:5 ω-3 (Usup et al., 2008). Similarly, in this study, although EPA and DHA were detected, there was an absence of the characteristic stearidonic and octadecapentaenoic acid. The proportion of palmitic, oleic and linoleic acid detected in the biochemical profile of *O. ovata* (Usup et al., 2008) was comparable to *Ostreopsis* sp. in this study, while EPA and DHA was approximately 6% lower in this study. Except for EPA content, fatty acid profiles and contents of the planktonic *Prorocentrum mexicanum* and *P. emarginatum* cultivated at 26 °C and a salinity of 36, but on a 14:10 photoperiod (Usup et al., 2008) were quite dissimilar to the one for the benthic *P. lima* in this study. Specifically, this study detected no C18:3 fatty acids, but amounts of DHA were 9-fold higher.

Benthic dinoflagellates in this study were separated by differences in total SFA, PUFA and MUFA. To the best of our knowledge, fatty acid profiles have not been conducted previously for *P. lima* and *G. carpenteri*, and therefore, it is difficult to put the observed differences in fatty acid compositions in perspective to other studies. A negative impact of low salinity and temperature on EPA content with no effect on DHA content was observed for *G. carpenteri*, while at normal salinity and low temperature, EPA contents remained unchanged, but DHA contents increased. For *Ostreopsis* sp. DHA and EPA contents were higher at reduced salinities at both temperatures. This can have flow-on effects in marine food webs, suggesting a higher nutritional value with regards to ω-3 LC-PUFA content in cooler and estuarine environments. It is possible that they are representative of normal fluctuations in the fatty acid profiles of these benthic dinoflagellates. More studies on the impact of geographic origin on

essential fatty acid profiles would be helpful to confirm the observations presented here.

In summary, population growth rates for *P. lima* were not affected by temperature or salinity, and potential geographic range expansion of this species could be a threat to oyster beds in NSW. By contrast, salinity and temperature reduced growth rates of *G. carpenteri* and *Ostreopsis* sp., respectively. The typical salinity levels and nutrient-rich waters at Merimbula, as well as the thermal tolerance of *G. carpenteri* indicate potential for populations to establish and proliferate in this southern locality. Although from a nutritional point of view, benthic dinoflagellates are a good source of EPA and DHA, it will be important to conduct further studies to investigate adaptability and acclimatization capability to confirm the true range expansion potential under climate change conditions. Nonetheless, the data presented here represent important baseline information for developing geographic range expansion models for these tropical potentially toxic benthic dinoflagellates.

Chapter Seven: Discussion

Ciguatera is a worldwide seafood-related illness caused by trophic transfer of ciguatoxins to mesopredators through the uptake of tropical benthic species of *Gambierdiscus* (Argyle et al., 2016; Chinain et al., 1999b; Kohli et al., 2014). The benthic dinoflagellate, *Gambierdiscus* is found in mixed assemblages with *Prorocentrum* and *Ostreopsis*, most frequently colonizing macroalgae (Morton and Faust, 1997; Parsons et al., 2011; Tester et al., 2014). Climate change is thought to support new colonization of substrates for macroalgae and associated benthic dinoflagellate assemblages in tropical regions, while it is hypothesised that the southward translocation of *Gambierdiscus* into temperate coastal habitats in NSW is due to a strengthened East Australian Current (EAC) (Heimann et al., 2011).

Health databases and questionnaires have previously only been used for epidemiological studies on reported ciguatera cases in tropical regions, including Queensland (Qld), Australia (Chateau-Degat et al., 2007; Chinain et al., 2010; Gillespie et al., 1986; Harvey, 1997; Rongo and van Woesik, 2011). The Qld government recognized the severity of ciguatera by inclusion of the illness in the foodborne disease database in 1987 (Harvey, 1997). Queensland coastal waters are the primary source of ciguatera in Australia, however, the distribution and ecophysiological responses of *Gambierdiscus* populations that potentially drive ciguatera occurrence is under-studied.

At the start of this PhD research, (which was conducted part-time), the state of knowledge was:

- Hales et al. (1999) reported a link between ciguatera occurrence and El Niño periods within south Pacific island nations;
- Chateau-Degat et al. (2005) demonstrated that following warmer sea surface temperatures (SSTs), (cause) a 13 to 17 month time line was needed for *Gambierdiscus* population to increase, leading to an increase in reported ciguatera cases (effect) three months later;
- Gillespie et al. (1985) haphazardly conducted a field study along the Qld coastline (1983 and 1984), excluding the central Great Barrier Reef (GBR) region, and found *Gambierdiscus toxicus* was present at low numbers across reef and coastal habitats, frequently co-occurring with *Prorocentrum* and *Ostreopsis* on most macroalgal substrates sampled. In a seasonal study on Flinders Reef, off south-east Qld, an increased abundance of *G. toxicus* was documented at 20 – 21 °C, in September 1983 and 1984.
- A link between an increase in *Gambierdiscus* population size with an increase in ciguatera incidence due to environmental and anthropogenic disturbances remained controversial;
- Likewise, substrate preference of *Gambierdiscus* populations for particular macroalgae remained conflicting;
- Prior to 1995, the now recognized 16 species of *Gambierdiscus* were all described as *G. toxicus*.

In summary, this thesis focused on knowledge gaps on conditions that govern occurrence of ciguatera in Qld, Australia. Specifically, it investigated the possible effects of:

- climate change and oceanic ecosystem trends;
- macroalgal substrate preferences of benthic dinoflagellate assemblages in the central GBR;
- population growth responses of GBR-isolates of *Gambierdiscus carpenteri* to temperature and salinity, and assemblage structure of associated benthic dinoflagellates; and
- impact of temperature and salinity on nutritional quality (essential long chain-polyunsaturated fatty acids (LC-PUFA), critical for marine food webs), nutrient uptake rates and growth of benthic dinoflagellates, including *G. carpenteri*.

Two drivers of ciguatera occurrence were identified in a desktop study (Chapter 2), through impacts on *Gambierdiscus* population dynamics: 1) climate and environment; and 2) trophic transfer and marine food webs (Figure 7.1).

7.1 Thesis Findings

7.1.1 Summary of research approach

An approach overview is illustrated in Figure 7.1, with drivers of ciguatera occurrence identified throughout the research. To ascertain current spatial and temporal distribution patterns, as well as the impact of climate on ciguatera occurrence in Qld, the government health database was sourced between 1996 and 2012 (Chapters 2 and 3). Prior to this research, this database was used for

epidemiological studies on ciguatera only. To investigate the effect of the multi-decadal phases of the Pacific Decadal Oscillation (PDO) on ciguatera occurrence, annual reported cases in this research (Chapter 3) was combined with previous studies by Gillespie et al. (1985) and Harvey (1997) from 1976 to 1995 (as records prior to 1976 were unreliable), to derive a 37-year continuous data set. This was fundamental to determine the effects of multi-decadal climatic conditions i.e. the ocean-scale PDO, on ciguatera incidence. To address the knowledge gap on benthic dinoflagellate assemblage composition and abundance in the central GBR, field studies were conducted at inshore and mid-reef sites (Chapter 4). When unobstructed by the presence of crocodiles or weather, i.e. cyclones, monthly field studies were conducted at Nelly Bay, Magnetic Island (19 ºS), during 2008 and 2009. Based on the highest *Gambierdiscus* population occurring in August at Nelly Bay, sampling was conducted in August 2009 at Pioneer Bay, Pelorus Island (inshore reefs), Lodestone and Keeper Reefs (mid reefs). To identify the potential impact on ciguatera occurrence under climate change scenarios, knowledge is needed on the interactive effect of predicted warmer SSTs with lower salinities experienced in coastal habitats in tropical and temperate regions. It was therefore essential to understand potential growth effects on *Gambierdiscus* and associated dinoflagellates. Controlled laboratory studies, were, therefore, conducted to ascertain the interactive effect of increased temperatures and lower salinities on population growth of benthic dinoflagellate assemblages, including *Gambierdiscus*, isolated from GBR waters (Chapter 5) and large-scale cultivation of benthic dinoflagellates was carried out to enable investigation of the impact of the interactive effect of lower temperature and salinity on

population growth, nutrient uptake and nutritional value for marine food webs (Chapter 6).

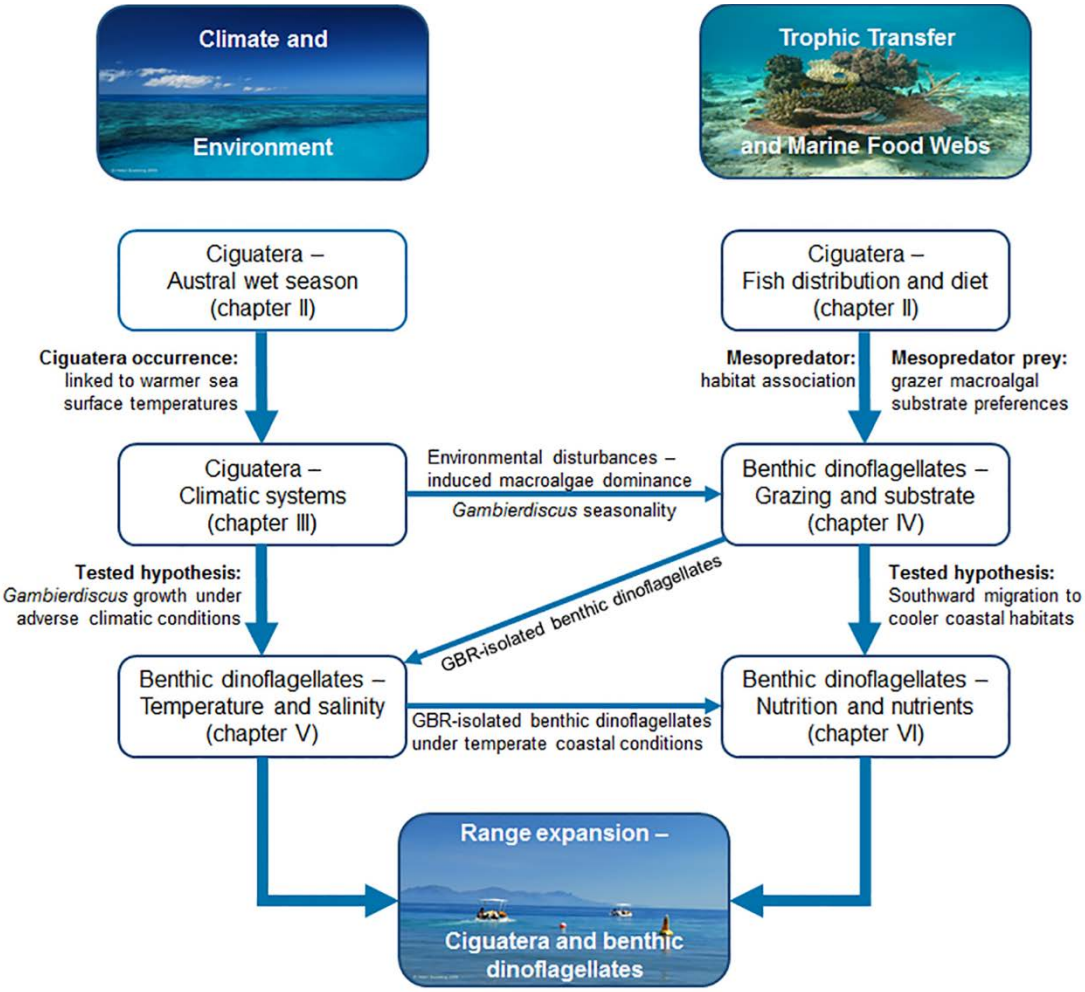

Figure 7.1 Flow diagram and alignment of PhD research conducted to identified and validate drivers that influence ciguatera occurrence

7.1.2 Drivers of ciguatera occurrence

Research presented in Chapter 2 suggested a regional effect in the spatial distribution trend of reported cases based on the geographic location of sufferers. This supported observations by Gillespie et al. (1986) that the number of reported cases decline with distance from metropolitan south-east Qld. This research was the first to investigate spatial trends based on the geographic

location of sufferers and demonstrated that the number of reported cases was lower in the central and far northern Qld regions, which have regionally isolated pockets of inhabitants between large uninhabited areas of national and state parks. This suggests that reporting of ciguatera cases is influenced by distance from metropolitan south-east Qld, and thereby, access to public health facilities. Distance and access to health facilities was also observed to affect reporting of ciguatera in the Pacific (Anderson and Lobel, 1987; Kaly et al., 1991). The limitations of estimating ciguatera incidence and frequency from health records based on reported cases is detailed in 7.2.

Spatial trends were also investigated based on the origin of fish (locality of fish catch) implicated in reported ciguatera cases. The three most frequent fish families implicated in ciguatera were Scombridae, Serranidae and Carangidae. Previous epidemiological studies on ciguatera in Qld identified Scombridae and Serranidae as most frequently implicated fish families in reported cases (Gillespie et al., 1986; Harvey, 1997). This PhD research delineated the southern end of the GBR (24 °S) as the boundary between higher frequency of cases associated with Serranidae in the north and Scombridae in the south. This supports a similar trend reported by Gillespie et al. (1986) and Harvey (1997), which identified the boundary at Yeppoon (23 ºS). The increase of Serranidae responsible for ciguatera from southern to northern regions, in this research, correlated with and evidenced their close association with reef habitat in the GBR. By contrast, contribution of Scombridae to ciguatera in the wet season and southern Qld, particularly Harvey Bay appeared to be governed by migratory patterns. Ciguatera cases caused by Carangidae was most frequent

in central Qld (Zone 3) and declined southwards. This research identified that movement habits of fish families may influence the regional frequency of association with ciguatera occurrence. The regional trend observed, based on location of ciguatera sufferers may be influenced by an increase of macroalgal substrates for *Gambierdiscus* populations to colonise following environmental disturbances, such as coral bleaching, cyclones and *A. planci* outbreaks, which occur frequently in the central GBR. Environmental disturbances were, therefore, investigated at a regional-scale in Chapter 3.

Chapter 2 demonstrated that ciguatera occurrence was 65% more frequent in the Austral wet season, suggesting a potential influence of warmer SSTs, characteristic for this season (Figure 7.1), which is additionally supported by reports on higher ciguatera frequency during the warmer wet season in French Polynesia between 1992 and 2001 (Chateau-Degat et al., 2007). The importance of climate and associated environmental disturbances, including climatic systems that induce warmer SSTs, was investigated at a broader-scale in chapter 3 (Figure 7.1). At the same time as this research, effects of PDO, Southern Oscillation Index (SOI) and El Niño on ciguatera occurrence was investigated in Pacific tropical regions (Llewellyn, 2010; Rongo and van Woesik, 2011), emphasizing the importance of the question investigated in Chapter 3, whether ocean-scale and multi-decadal climate periods are linked to ciguatera frequency in Qld.

Chapter 3 adopted a model-based approach to determine whether trends identified in Chapter 2 were linked drivers of ciguatera incidence. The multi-

decadal PDO significantly influenced the frequency of ciguatera occurrence in Qld. The long-term, continuous dataset, synthesized from available public data in this research, demonstrated 62 – 85% higher ciguatera occurrence during the warm PDO phase, compared to the cool PDO phase. This has significant implications for predictions of likely periods of increased ciguatera occurrence under climate change conditions, i.e. effects of the imminent shift to the PDO warm phase. Studies, during the time of this research, identified a link between ciguatera and the PDO in the Pacific, however, while it is noted that the studies were conducted within the same PDO phases as this research, the datasets were limited to the warm phase (1977 – 1998) (Llewellyn, 2010) and the cool phase (1999 – current) (Rongo and van Woesik, 2011). Likely outcomes with regards to ciguatera incidence in Qld can, therefore, not be extrapolated from these studies alone.

This research determined that the PDO at 2-year lag time was the best climate model, with 54% support followed by the PDO at zero and 1-year lag time. An influence of the PDO was also demonstrated for several Pacific island nations (Llewellyn, 2010), particularly, Kiribati, Fiji and Vanuatu showed a positive link to ciguatera with the PDO at a 2-year lag time, with 41, 49 and 51% support, respectively (Llewellyn, 2010). Climate models based on the SOI and El Niño did not support an effect on ciguatera incidence for Qld, while similar SOI- and El Niño- based models showed significance for the Pacific (Llewellyn, 2010; Rongo and van Woesik, 2011). It is hypothesised that the no effect of the SOI and El Niño on ciguatera incidence in this research was masked by a latitudinal effect, as the length of the Qld coastline spans almost 18° of latitude, which

would also explain regional differences in trends identified in Chapter 2. This hypothesis is supported by opposing model outcomes for New Caledonia and French Polynesia (21 and 18 °S, respectively) to other Pacific Island nations at 3 – 18 °S latitude (Llewellyn, 2010). Similarly, latitudinal spread of the Cook Islands was hypothesised as being responsible for differences in frequency of ciguatera between the northern and southern Cook Islands (Rongo et al., 2009).

The best climate model, PDO at 2-year lag time, also demonstrated that ciguatera occurrence declines in years when coral bleaching is occurring. This supports the model outcome reported by Chateau-Degat et al. (2005), which established a 16 – 20 month time lag between cause (warmer SSTs) and effect (increased ciguatera occurrence). It is noteworthy that the modelling research conducted here is the first to support Chateau-Degat et al. (2005) model outcome, and no differing outcomes have yet been reported. Climate change scenarios predict warmer SSTs will increase the frequency of coral bleaching in tropical regions, and strengthen the EAC, to extend warmer SSTs along the east Australian coastline towards Tasmania (Heimann et al., 2011). Environmental disturbances are thought to lead to periodic increases in ciguatera due to a flow-on effect of increased macroalgal dominance on reefs, offering ideal substrates for benthic dinoflagellates to colonise and subsequent increased transfer of ciguatoxins into marine food webs (Figure 7.1).

Research in Chapter 3 identified environmental disturbances to have the highest impact on coral cover in the central GBR, a region characterised by most frequent occurrence of severe tropical cyclones, coral bleaching and the

majority of *A. planci* outbreaks. The more frequent occurrence of environmental disturbances in the central GBR is likely to support a greater range of macroalgal substrates and associated benthic dinoflagellates, but the central GBR region was excluded in the only reported survey on *Gambierdiscus* abundances in the GBR by Gillespie et al. (1985). In Chapter 4, field research was conducted at inshore and mid-shelf reefs in the central GBR to address the knowledge gap of temporal abundance, composition of benthic dinoflagellate assemblages, and macroalgal substrate preferences by dinoflagellates. Although co-occurrence with *Prorocentrum* and *Ostreopsis* was noted by Gillespie et al. (1985), composition of benthic dinoflagellate assemblages and seasonal abundance for benthic dinoflagellates was not investigated on the GBR. Similar to reports by Gillespie et al. (1985), *Gambierdiscus* was a minor component of benthic dinoflagellate assemblages at inshore and mid-shelf reefs. Dominance of *Ostreopsis* at Nelly Bay corroborated observations by Gillespie et al. (1985) for northern and southern GBR, however, *Prorocentrum* dominated benthic dinoflagellate assemblages at Pioneer Bay, Pelorus Island, Lodestone and Keeper Reefs. The dominance of *Prorocentrum* in benthic dinoflagellate assemblages in November 2008 at mid-shelf reefs, Bramble, John Brewer, and Trunk Reefs, and dominance of *Ostreopsis* at inshore reefs on Pelorus Island (Heimann et al., 2010) suggest regional and potentially reef-related environmental factors are likely to influence the composition of benthic dinoflagellate assemblages. While *Gambierdiscus* abundance field studies have been conducted for Pacific Islands, benthic dinoflagellate assemblage composition was not investigated, making a comparison to the results obtained here impossible.

The distribution of *Gambierdiscus* on macroalgal substrates have been reported as patchy within reefs and highly variable among reefs when substrate preference was investigated using phyla or defence strategies against grazing as categories (Ballantine et al., 1985; Bomber et al., 1988b; Faust, 1995; Nakahara et al., 1996; Parsons et al., 2011). Parsons et al. (2011) directly tested substrate preference under laboratory-controlled conditions and although there was no clear substrate preference, the use of macroalgal surfaces as an anchor point and differential levels of motility by *Gambierdiscus* among substrates supported earlier observations. Often a single macroalgal phylum dominated study sites during the field studies (Chapter 4), while thallus structure (i.e. terete, flattened, leathery) was more diverse. It was therefore hypothesised that surface structure may be more important in explaining differences in *Gambierdiscus* abundances than taxonomic diversity. This research, therefore, used a novel approach that explored the use of macroalgae surfaces as anchor points and investigated macroalgal substrate preference by thallus structure. A non-selective preference for substrate was established in this research, as preference varied among reefs and between inshore and mid-shelf reefs. This research corroborates the low abundances of *Gambierdiscus* recorded by Gillespie et al. (1985) across seasons in northern and southern GBR with no clear macroalgal substrate preference of *Gambierdiscus* identifiable. The variability in macroalgal colonisation by *Gambierdiscus* documented in this research may indicate that availability of macroalgal substrates are likely to govern colonisation patterns and population size. If this hypothesis is correct, then the question of macroalgal substratum preference by

Gambierdiscus would only be resolved at sites with high diversity of macroalgae (Figure 7.2). As *Gambierdiscus* utilises macroalgal surfaces as anchorage points, colonisation of specific macroalgal substrates is also likely influenced by environmental conditions such as light, currents, temperature, salinity and nutrient supply (Figure 7.2).

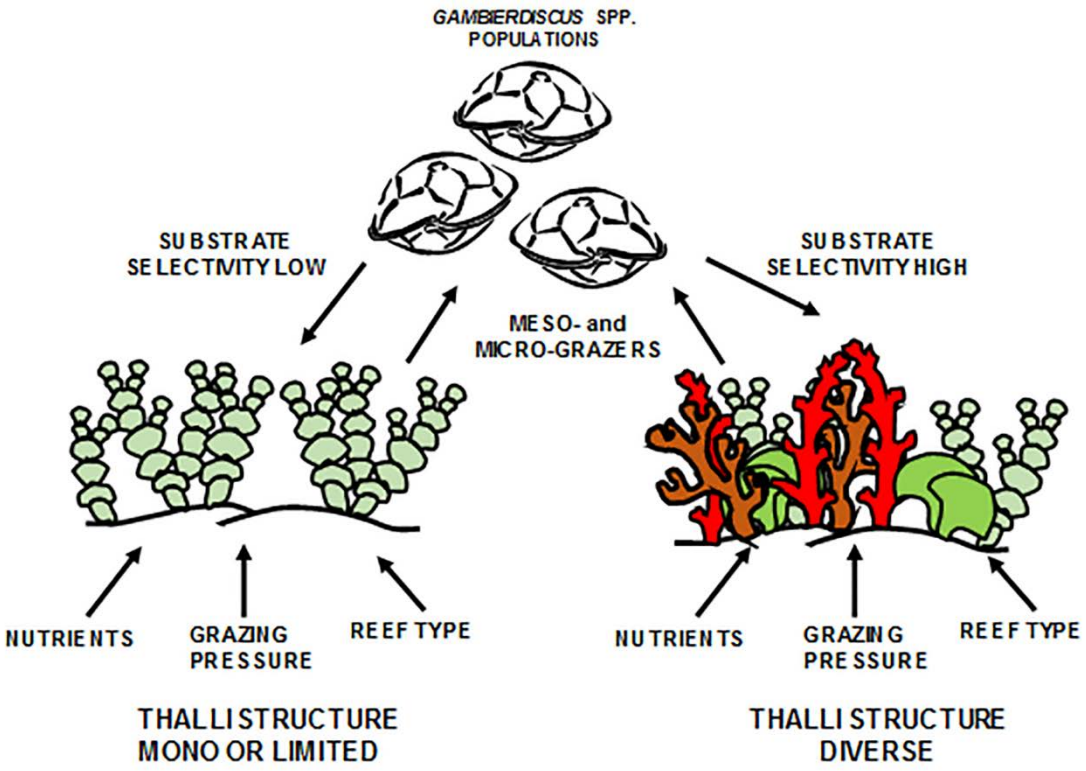

Figure 7.2 The influence of macroalgal thallus structure diversity and reef-specific environmental conditions on *Gambierdiscus* substrata selectivity.

Chapter 2 identified that dietary components (fish, benthic crustaceans, and other invertebrates) were relatively consistent in mesopredators frequently implicated in ciguatera. A second novel approach to investigate macroalgal substrate preference, was therefore, tested based on macroalgal palatability for grazers (Chapter 4). Abundances for benthic dinoflagellate assemblages varied among reefs with substrates palatable to invertebrates often higher than fish palatable substrates. Differences between abundance of benthic dinoflagellate

assemblages and *Gambierdiscus* was observed when grazer palatability was categorised by thallus structure. A herbivory fish *in situ* exclusion experiment conducted at Orpheus Island demonstrated the increased abundance and dominance of leathery macroalgae of the genus, *Sargassum* (Hughes et al., 2007). Similarly, *Sargassum* dominance at Nelly Bay may suggest low grazing pressure by herbivorous fish, and whether this permits the dominance of other grazers such as sea urchins is unknown. The abundance of *Gambierdiscus* and benthic dinoflagellate assemblages documented in this research provides valuable information for studies of grazer assemblages, which are currently not available (Figure 7.2).

The consistent low abundances of *Gambierdiscus* on the GBR all year round and non-selectivity for macroalgal substrate would assist EAC-transported *Gambierdiscus* in colonization of macroalgal surfaces in southern NSW coastal habitats (Figure 7.1). Chapter 2 identified a spatial trend in the distribution of ciguatera in Qld, which suggests a potential broad tolerance range of *Gambierdiscus* to temperatures and salinities. Tolerance for lower temperatures was reported in a seasonal study conducted by Gillespie et al. (1985) at Flinders Reef (27 ºS) in south-east Qld, who observed increased abundances of *Gambierdiscus* at temperatures below 22 ºC. Sampling following a heavy rain-induced freshwater plume in March in the seasonal study conducted in the central GBR (Chapter 4) documented lower abundances of benthic dinoflagellates (Heimann et al., 2010), suggesting that the regional *Gambierdiscus* populations and associated dinoflagellate assemblages are sensitive to low salinity. As in Chapter 3, environmental disturbances were

identified as a potential driver of ciguatera occurrence and GBR-derived benthic dinoflagellate cultures were established in Chapter 4, Chapters 5 and 6 could now test directly the effect of temperature, salinity and nutrients on population growth of GBR – isolated benthic dinoflagellates (Figure 7.1).

As environmental disturbances can lower salinities during periods of warmer temperatures, the interactive effect of temperature and salinity was investigated in chapter 5 using the GBR – isolated benthic dinoflagellates. This identified a significant effect of temperature and lower salinities but no interaction on population growth for two *G. carpenteri* strains isolated from different habitats but at the same latitude, which suggest strongly that habitat history is important in acclimating to adverse environmental conditions and expansion to southern NSW coastal habitats. Along with the determination of *Gambierdiscus* as a species-rich genus, these data support first reports that differences in environmental tolerances of *Gambierdiscus* were geographically influenced (Kibler et al., 2012; Xu et al., 2016).

In Chapter 4, *Gambierdiscus* populations co-occurred with other benthic dinoflagellates. To test whether co-occurring benthic dinoflagellates had an effect on population growth of *G. carpenteri*, cells were grown in a mixed assemblage of *P. lima* and *Ostreopsis* sp. and at low salinities, that naturally occur in coastal habitats (Chapter 5). The effect of species-interaction varied at different salinities and dominance of assemblage. Growth of *G. carpenteri* was inhibited at a salinity of 36 by mixed assemblage, regardless of *P. lima* or

Ostreopsis sp., dominance, but growth patterns were indicative of recovery at a salinity of 16 when *Ostreopsis* sp. dominated the cultures. This research is the first to investigate the effect of environmental parameters and assemblage composition on the population growth of *G. carpenteri*.

Populations of *Gambierdiscus* have been recently recorded along the New South Wales (NSW) coastline (32 – 37 °S), including *G. carpenteri* in southern waters off Merimbula (37 °S) (Ajani et al., 2013; Kohli et al., 2014). This suggests the potential southward geographic expansion of *Gambierdiscus*, transported by the EAC, from the GBR to southern NSW coastal habitats (Figure 7.1). The nutritional value of benthic dinoflagellates, including *Gambierdiscus* may influence whether they would be targeted by resident fish assemblages in cooler southern waters. Coastal habitats are characterised by variable environmental conditions, particularly temperature and salinity. The effect of environmental conditions, however, on nutrient availability and uptake rates, which determine population growth rates, and the nutritional value of benthic dinoflagellate cultures has not, to date, been investigated. Therefore, the effect of temperature and salinity on nutritional value, nutrient uptake and population growth, using large-scale cultures of *G. carpenteri*, *P. lima* and *Ostreopsis* sp. was investigated in Chapter 6.

Nutrient uptake rates in this research, were influenced significantly by species.This may explain the generally species-specific temperature and salinity preferences. The oligotrophic waters of the GBR (Furnas et al., 2005)

would sustain small populations of benthic dinoflagellates, based on calculated nutrient consumption rates in Chapter 6. This maintains the generally low abundances of benthic dinoflagellates documented on the GBR in this research (Chapter 4) and reported by Gillespie et al. (1985) and Heimann et al. (2010). By contrast, nutrient-rich temperate waters in NSW (Scanes et al., 2007) and the Mediterranean (Accoroni et al., 2017) are likely to exceed growth requirements for maintaining large benthic dinoflagellate populations that could reach millions of cells per litre. Such bloom development was observed recently in the Mediterranean with *Ostreopsis ovata* (Accoroni et al., 2011) and with the high seasonal abundances of *G. carpenteri* in Merimbula, NSW (Kohli et al., 2014).

Population growth of *P. lima* was not significantly affected by temperature or salinity, and as this species has been implicated in diarrhetic shellfish poisoning, the potential geographic expansion could increase the threat to the NSW shellfish industry under climate change scenarios. By contrast, salinity and temperature challenged growth of *G. carpenteri* and *Ostreopsis* sp., respectively. The growth rate for *G. carpenteri* was linked to nutrient uptake, which was higher at a salinity of 36 in both temperatures. At the higher growth rate, the essential omega-3 fatty acid, docosahexaenoic acid (DHA) increased at 24 °C and a salinity of 36. By contrast, the low growth rates for *Ostreopsis* sp. at 24 °C in both salinities were linked to higher nitrogen and phosphate rates, indicating stress-induced consumption, and potential production of osmolytes as a physiological response for survival (Rosic et al., 2011; von Alvensleben et al., 2016). Essential omega-3 fatty acid contents for *Ostreopsis* sp., however, were

higher at low salinities at both temperatures. This research established the likely higher nutritional value of benthic dinoflagellates in temperate coastal habitats, and therefore, the potential for them to be targeted by temperate marine fish species. Additionally, this research established the potential for *G. carpenteri* and *Ostreopsis* sp. populations to establish and proliferate in southern geographic locations, such as Merimbula, NSW, which have relatively stable salinity levels and nutrient-rich waters.

7.2 Limitation of this research and future research foci

While the use of a public health database for desktop studies on ciguatera occurrence in this research identified potential spatial and temporal trends, the underlying influences, such as human lifestyle, diet choices, frequency of fish consumed and availability and source of fish species was not revealed. In addition, habitat association of fish species implicated in ciguatera can also not be unravelled based on information in health databases and records alone. Further, as health records do not enable evaluation of the impact of under-reporting, identifying significant trends remains challenging. To overcome at least the human population-associated limitations, construction of a meaningful questionnaire would offer a simple approach to obtain substantive quantitative and qualitative data that can evaluate the potential impact of under-reporting of ciguatera and whether the rate of under-reporting increases with geographic isolation. This was already suggested by Lewis et al. (1988) who identified that extensive questionnaires are needed to provide more accurate estimates on prevalence and distribution of ciguatera in Qld. In the continued absence of such questionnaires, the impact of ciguatera on coastal populations and the

socio-economic costs to commercial fisheries in Qld will remain a limitation in the management of ciguatera.

While high abundances of benthic dinoflagellates can be recorded in the field, difficulties with field-based studies include timing, costs, and labour intensive sampling. To investigate environmental effects on benthic dinoflagellate populations *in situ*, requires at least 5 years of continuous data and, therefore, are not sustainable. Low abundances of *Gambierdiscus* on the GBR all year round, but continued reports of ciguatera incidence documented in this research, in addition to the demonstrated high nutritive long chain polyunsaturated omega-3 fatty acids, indicate active grazing pressure and ready transfer of the toxins via the food web. This research identified the need for field surveys on fish assemblages and grazing of macroalgal substrata. Fish surveys utilising video technology enables extensive data to be collected with an efficient and cost-effective use of resources (Mantyka and Bellwood, 2007). Data obtained from field surveys on fish grazing assemblages could then be applied to much needed feeding trials that simulate natural marine food webs. Initial lack of all dinoflagellate cultures made feeding experiments impossible, however, as a result of this research, isolates are now available to enable the growth of large-scale cultures required for feeding trials possible.

Compounding factors in the field can be overcome by controlled laboratory studies if benthic dinoflagellates can be domesticated. Although not described in detail in this thesis, it is worth noting that prior to controlled laboratory

experiments, domestication of benthic dinoflagellate genera proved rather difficult. Cultured microalgae, in general, can be maintained under similar conditions, including media, temperature and light as well as aeration in large-scale cultures, which allow sampling to be easily homogenised for counting and analysis. These culture conditions proved non-suitable for benthic dinoflagellates, and although literature reported culture growth in commonly used media, i.e. f/2, *Gambierdiscus* isolates from the GBR only survived in K – medium. In addition, disturbance to flasks, or homogenising cultures caused GBR-derived benthic dinoflagellate cells to stress and lyse. Therefore, the isolation, establishment and maintenance of monocultures for GBR-derived benthic dinoflagellates required a trial and error approach, in which more than 12 months were needed to successfully achieve domestication.

One of the main hurdles in sampling field populations and establishing cultures is the accurate identification of benthic dinoflagellate genera, which is currently time consuming and costly, including light microscopy and scanning electron images supported by genetic analysis. Alternative methods of identification are needed to be explored, of which the tree of life approach may be a suitable alternative. Barcoding of the cytochrome oxidase 1 (CO1) is informative for dinoflagellates (Stern et al., 2012), however, this is yet to be established for benthic dinoflagellates involved in this research. While this will initially require extensive sampling and laboratory work to create the barcoding for benthic dinoflagellates, in the long term it is likely to offer a more efficient method for use in the field.

As discussed above, the governance of the multi-decadal PDO on ciguatera occurrence was documented in this research. It is predicted, based on this research that an increase in ciguatera outbreaks and the potential for proliferation of *Gambierdiscus* with its southward geographic expansion will occur following the imminent transition into the next PDO warm phase. Investigation of climate and environmental disturbances on ciguatera occurrence in Qld was limited by the extensive coastline of Qld, which spans more than 17 º of latitude, essentially spanning the tropics. Latitudinal impacts were also demonstrated for Pacific regions, which influenced outcomes of climatic events (Llewellyn, 2010; Rongo et al., 2009). The latitudinal distance of the Qld coastline and the unknown effect of under-reporting of ciguatera, particularly in northern Qld, potentially masked links of ciguatera with the SOI and El Niño periods. Spatial and temporal distribution patterns at a regional-scale were impeded by latitudinal distance of the Qld coastline, patchy investigations on the abundance and distribution of benthic dinoflagellate assemblages, including *Gambierdiscus*, migratory patterns of mesopredators implicated in ciguatera, and limited knowledge on the impact of environmental disturbances on coral reef food webs. The limited available data, particularly at a regional-scale, hindered establishment of baseline information that could be applied to model-based investigations and predictions of ciguatera hotspots under climate change scenarios. To overcome these limitations, long-term interrelated studies along the Qld coastline, including macroalgal diversity, *Gambierdiscus* and co-occurring benthic dinoflagellate abundances, fish diets and a better understanding of food webs is essential. This needs to be co-ordinated with regional-scale questionnaires on the incidence of ciguatera in

human populations. Initial sampling efforts need to be sustained over at least 5 – 10 years, due to the lag of time between the cause (climate) and effect (ciguatera outbreaks) documented in this research. The huge sampling effort needed to obtain this knowledge would be cost prohibitive and beyond the scope of a doctorate research study. Sampling effort would need to be directed to identified regional trouble areas and then extrapolated or modelled. In southern Qld, Platypus Bay, Fraser Island (Harvey Bay) is a globally known ciguatera hotspot; while extensive freshwater plumes, which lower salinities and are a rich source of nutrients, often impact reef health of the inshore Keppel Islands. As the growth of *G. carpenteri* is linked to nutrient uptake, these reefs could potentially be another southern regional ciguatoxic hotspot. Trunk and Lodestone Reefs in central Qld were identified as areas of concern by this research (Chapter 2), while areas of concern in northern Qld are unknown. Questionnaires on ciguatera incidence in northern Qld regional populations are needed, so that local knowledge on reefs of regional concern can be identified before concentrating regional sampling effort.

Linking of the dataset derived in this research with other large datasets on water quality parameters, ocean and coastal currents, climate, coral bleaching and *Acanthaster planci* (crown-of-thorns starfish) outbreaks, which have been collated by stakeholders in reef and human health (including the Australian Institute of Marine Science (AIMS), CSIRO and The Centre for Tropical Water and Aquatic Ecosystem Research (TropWATER)), could assist in further determining likely regional hotspots for ciguatoxin trophic transfer using model-based investigations.

7.3 Conclusions

This research has addressed the principle aim to provide a better understanding of the potential drivers of ciguatera occurrence and the effect of predicted climate change on the potential range expansion of benthic dinoflagellates into new geographic regions. Two drivers of ciguatera occurrence were identified: 1) climate and environmental disturbances; 2) trophic transfer and marine food webs. In addition to the identification of two drivers of ciguatera occurrence, the findings of this research:

- Contributed to the understanding of ciguatera in Qld;
- Demonstrated governance of multi-decadal climate and linked with coral bleaching;
- Enabled base line information to model predictions; and
- Established that southward geographic expansion into NSW coastal habitats has potential based on:
    - The non-selectivity of substrates;
    - Population growth responses to environmental conditions; and
    - The high nutritional value of benthic dinoflagellates for grazing fish and invertebrates.

This now lays solid foundation for studies to be given priority in ciguatera research.

In conclusion, the identified influence of the multi-decadal PDO has implications for management in southward migration of benthic dinoflagellates and fish species at high ciguateric risk, such as Spanish mackerel; a time-line with lower ciguatera incidence in the year of coral bleaching with a two-year time lag to a periodic increase; benthic dinoflagellate responses to different environmental

conditions varies between genera; and that benthic dinoflagellate assemblages can improve survival of *G. carpenteri* in lower salinities. This research established that spatial and temporal trends require regional-scale field research supported by controlled laboratory experiments and extensive questionnaires. It has been demonstrated that habitat history may influence the adaptability and acclimatisation of benthic dinoflagellates to new geographic locations, thereby, influencing their success for southward geographic expansion and colonisation in NSW coastal habitats; potentially supported by non-substrate preference of *Gambierdiscus* in the central GBR. Low abundances all year round in addition to the demonstrated high nutritive long chain polyunsaturated omega-3 fatty acids indicate active grazing pressure and ready transfer of the toxins via the food web.

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Appendix A

Chapter One:

Supplementary Table S1.1. Frequency and occurrence of ciguatera hot spots, reported ciguatera cases acquired through affordable travel, fish exports and local fish caught outside tropical regions, expansion of *Gambierdiscus* spp. recorded in temperate regions and expansions within tropical regions.

Tropical regions				
Hot spots	**Total no. cases (no. years)**	**Average no. cases / 100,000**	**Total no. years**	**References**
Cook Islands	2687	1437	11	(Skinner et al., 2011)
French Polynesia	8534	329	11	(Skinner et al., 2011)
Kiribati	3183	313	11	(Skinner et al., 2011)
Marshall Islands	2122	416	11	
Niue	50	303	11	
Tokelau	279	1576	11	
Vanuatu	8127	397	11	
Antigua & Barbuda	4548	6527	15	(Caribbean Epidemiology Centre, 2008)
Bahamas	1888	741	15	
British Virgin Is.	985	5541	15	
Cayman Islands	361	1214	15	
Jamaica	357	15	15	
Montserrat	571	6624	15	
Travel location	**Implicated fish**	**Travel originated**	**Year**	
Florida, USA	Barracuda	Brazil	1972	(Barkin, 1974a)
Cuba	Unknown	Canada	1987	(Frenette et al., 1988)
West Indies	Coral Trout	France	1994	(Moulignier et al., 1995)
Haiti	Amberjack	USA	1995	(Poli et al., 1997)
Cuba	Barracuda	Italy	1999	(Butera et al., 2000)
Pacific Islands	Grouper	Hong Kong	2004	(Cheng and Chung, 2004)
Pacific Islands	Grouper	China	2004	(Chan, 2014)
St Eustatius	Snapper	England	2004	(Kipping et al., 2006)
Pacific Islands	Grouper	China	2005	(Chan, 2014)
Pacific Islands	Snapper & Grouper	Hong Kong	1989-2008	(Chan, 2014)
Mexico	Local seafood	Qld, Australia	Pre-2008	(Slobbe et al., 2008)
Qld, Australia	Barracuda	Unknown	Pre-2008	(Slobbe et al., 2008)

Temperate regions				
Import location	**Exported fish**	**Location source**	**Year**	**References**
Toronto, Canada	Barracuda	Jamaica	1983	(Todd, 1997)
Ontario, Canada	Grouper	USA	1984	(Todd, 1997)
NSW, Australia	Spanish mackerel	Hervey Bay, Qld	1987	(Sydney Fish Markets, 2005)
Ontario, Canada	Grouper	Spain	1989	(Todd, 1997)
Quebec, Canada	Mahi-mahi	USA	1989	(Todd, 1997)
San Francisco, USA	Barracuda	Florida, USA	1989	(Geller et al., 1991)
Vancouver, British Columbia	Grouper	Fiji	1990	(Todd, 1997)
Toronto, Canada	Barracuda	USA	1993	(Todd, 1997)
Toronto, Canada	Barracuda	Trinidad & Tobago	1997	(Todd, 1997)
Toronto, Canada	Mackerel	West Indies	1997	(Todd, 1997)
Import location	**Exported fish**	**Location source**	**Year**	
Victoria, Australia	Maori Wrasse	Queensland	1997	(Ng and Gregory, 2000)
North Carolina, USA	Amberjack	Florida, USA	2007	(Langley et al., 2009)
St Louis, Missouri, USA	Amberjack	Louisiana, USA	2007	www.yourlawyer.com
New York, USA	Grouper & Barracuda	Florida, USA	2011	(Graber et al., 2013)
Canada	Grouper & Barracuda	Cuba	1983-1997	(Todd, 1997)
Paris, France	unknown	West Indies	Pre-1995	(Moulignier et al., 1995)
Implicated fish	**Location**	**Country**	**Year**	
Barracuda	North Carolina	USA	1987	(Morris et al., 1990)
Barracuda & Snapper	Texas	USA	1998	(Villareal et al., 2006)
Amberjack	Canary Islands	Spain	2004	(Pérez-Arellano et al., 2005)
Barracuda	South Carolina	USA	2004	(Villareal et al., 2006)
Amberjack	Canary Islands	Spain	2012	(Nunez et al., 2012)
Reef fish	Okinawa	Japan	1997-2008	(Oshiro et al., 2010)
Amberjack	Canary Islands	Spain	2008-09	(Boada et al., 2010)

Note: *G. yasumotoi* re-classified, now *Fukuyoa paulensis*

Appendix B

Chapter Two:

Supplementary Table S2.1. Reported ciguatera cases in Queensland, between 2001 and 2012, including available data on fish species; location fish originated, including regional zone; source of fish, purchased or recreationally caught, with percentage calculations detailed below the Table.

YEAR	QUARTER	MONTH	REGION	LOCATION	SITE #	ZONE	# CASES	FISH (common name)	SCOMBRID	SERRANIDAE	OTHER	PURCHASED	RECREATIONAL
2001	Jan-Mar	January					14	Spanish mackerel	14				
2001	Jan-Mar	February					2	Spotted mackerel	2				
2001	Apr-Jun	June					3	Barracuda			3		
2001	Oct-Dec	November					4	Coral trout		4			
2001	Oct-Dec	November					9	Spanish mackerel	9				
2002	Jan-Mar	February					2	Striped perch			2		
2002	Jan-Mar	March					2	Spanish mackerel	2				
2002	Apr-Jun	April					3	Grunter Bream			3		
2002	Oct-Dec						7	unknown					
2003	Jan-Mar	January					2	Coral trout		2			
2003	Jan-Mar	January					3	Mackerel	3				
2003	Jan-Mar	February					7	Coral trout		7			
2003	Jan-Mar	March	1	Mooloolaba Bay	3	1	3	unknown					
2003	Apr-Jun	May					2	Cod		2			
2003	Apr-Jun	May					3	Giant trevally			3		
2003	Jul-Sept	August					5	Barracuda			5		
2003	Oct-Dec	October					15	Spanish mackerel	15			15	
2003	Oct-Dec	November					3	Red emperor			3		
2003	Oct-Dec	December					4	unknown					
2004	Jan-Mar	January					2	Golden spotted trevally			2		
2004	Jan-Mar	February					4	Coral trout		4		4	
2004	Jan-Mar	March					2	unknown					
2004	Apr-Jun	April					5	Spanish mackerel	5				
2004	Apr-Jun	June					3	Trevally			3	3	
2004	Apr-Jun	June					4	Grey mackerel	4				
2004	Jul-Sept	July					4	Grey mackerel	4				
2005	Jan-Mar	January					4	Mackerel	4				
2005	Jan-Mar	January					2	Black trevally			2		
2005	Jan-Mar	March					2	Yellowtail kingfish			2		
2005	Apr-Jun	April	2	Hervey Bay	17	2	17	Spanish mackerel	17			17	
2005	Jul-Sept	August					2	Spanish mackerel	2			2	
2005	Jul-Sept	September					2	Trevally			2	2	
2005	Jul-Sept	September					5	Black kingfish			5	5	
2005	Oct-Dec	December					10	Barracuda			10	10	
2005	Oct-Dec	December	2	Hervey Bay	8	2	8	Yellowtail kingfish			8		8
2006	Jan-Mar	February	2	Gladstone	2	2	2	Cod		2			2
2006	Jan-Mar	March					2	Trevally			2	2	
2006	Jan-Mar	March					4	Spanish mackerel	4			4	
2006	Jul-Sept	July					2	Spanish mackerel	2				
2006	Oct-Dec	October					4	Black kingfish			4		
2007	Jan-Mar	February					2	Mackerel	2				
2007	Jan-Mar	March					6	Mackerel	6				
2007	Apr-Jun	May	5	Cairns	3	5	3	Coral trout		3		3	
2007	Apr-Jun	May	2	Hervey Bay	2	2	2	Mackerel	2			2	
2007	Jul-Sept	September					5	Coral trout		5			
2007	Oct-Dec	November	2	Gladstone	2	2	2	Coral trout		2		2	
2007	Oct-Dec	December	5	Upolu, Vlasoff & Michaelmas Cays	2	5	2	Spanish mackerel	2				2
2008	Jan-Mar	March					6	Black kingfish			6		6
2008	Jan-Mar	March	1	Brisbane	2	1	2	Yellowtail kingfish			2	2	
2008	Jul-Sept	July	3	Saumarez Reef	4	3	4	Yellowtail kingfish			4	4	
2008	Jul-Sept	July	1	Brisbane	6	1	6	Red emperor			6	6	
2008	Oct-Dec	December	4	Trunk Reef	3	4	3	Cod		3			
2009	Jan-Mar	February	1	Mooloolaba	3	1	3	Spanish mackerel	3			3	
2009	Jul-Sept	August	1	Capel Bank, Brisbane	2	2	2	King snapper			2	2	
2009	Jul-Sept	July	2	Bundaberg	2	2	2	Reef cod		2			2
2010	Jan-Mar	January	1	Bribie Island	4	1	4	Spanish mackerel	4			4	
2010	Jan-Mar	January	1	Brisbane	6	1	6	unknown				6	
2010	Jul-Sept	July	4	Townsville	2	4	2	Spanish mackerel	2			2	
2010	Jul-Sept	August	3	Mackay	2	3	2	Coral trout		2		2	
2010	Jul-Sept	August	1	Brisbane	4	1	4	unknown				4	
2010	Oct-Dec	November	4	Lodestone Reef	4	4	4	Passionfruit trout		4			4
2010	Oct-Dec	December					2	Mangrove Jack			2	2	
2011	Jan-Mar	March	4	Reef off Lucinda	3	4	3	Red bass			3		3
2011	Jul-Sept	July					3	unknown					3
2011	Jul-Sept	August					3	Coral trout		3			
2011	Oct-Dec	November					6	Spanish mackerel	6				
2011	Oct-Dec	December					2	Coral trout		2			
2012	Jul-Sept	August					2	Coral trout		2			
2012	Jul-Sept	September					2	Coral trout		2			
TOTAL					**86**		**278**		**114**	**51**	**84**	**108**	**30**

2005+ 161
southern 67
northern 19

From 278 cases between 2001 and 2012:

A. 249 cases included fish species (89.57%)
of these 114 cases were scrombids ie. Mackerels (45.78%)
51 cases were serranids i.e. Coral trouts and cods (20.48%)
84 cases were other species (33.73%)

B. 138 cases included source i.e. method of attainment (49.64%)
of these 108 cases were purchased i.e. restaurant or shop (78.26%)
30 cases were recreationally caught fish (21.74%)

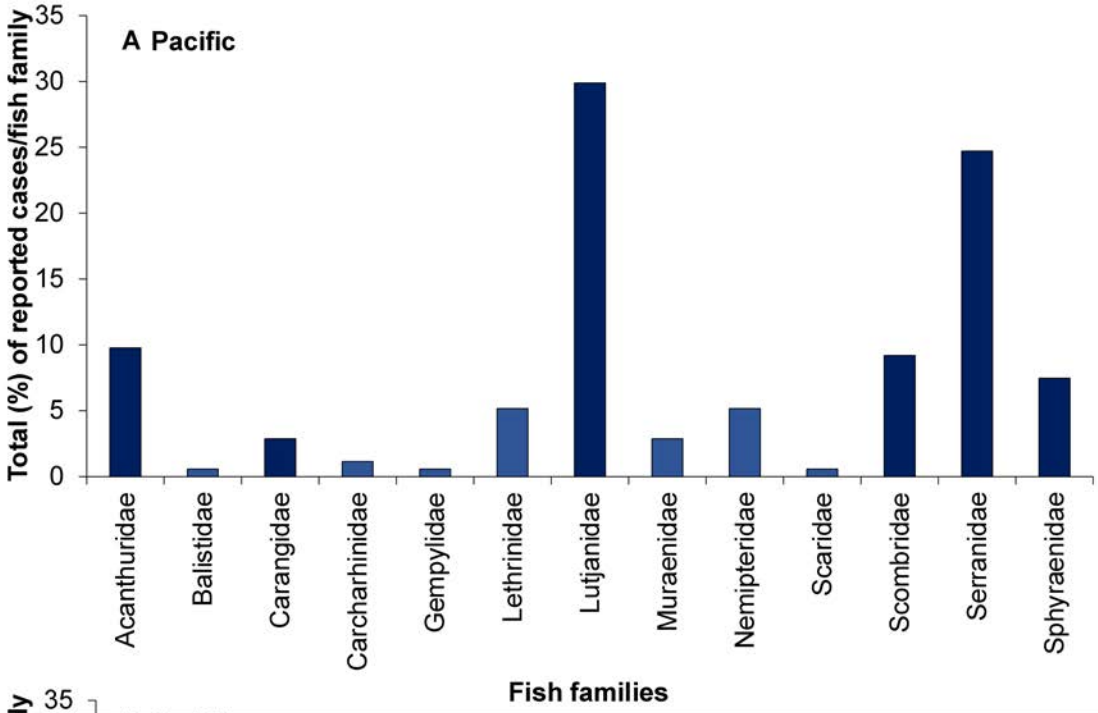
C. 86 cases included location fish was sourced (30.94%)
however, location not incl prior to 2005 i.e. total in 5yr period 161 cases (51.55%) with exception of one incident in 2003
19 cases were northern Qld i.e.north of Rockhampton (22.89%)
64 cases were southern Qld (77.11%) and 67 incl 2003 (77.91%)
three distinct regions in the south:
Rockhampton to Hervey Bay with 12 cases (18.2%) and incl 2003 incident (17.39%)
Hervey Bay i.e. incl hotspot being Fraser Island with 27 cases (40.9%) and incl 2003 incident (39.13%)
Southeast region being south of Fraser Island i.e. Noosa to Brisbane region with 27 cases (40.9%) and incl 2003 with 30 cases (43.48%)

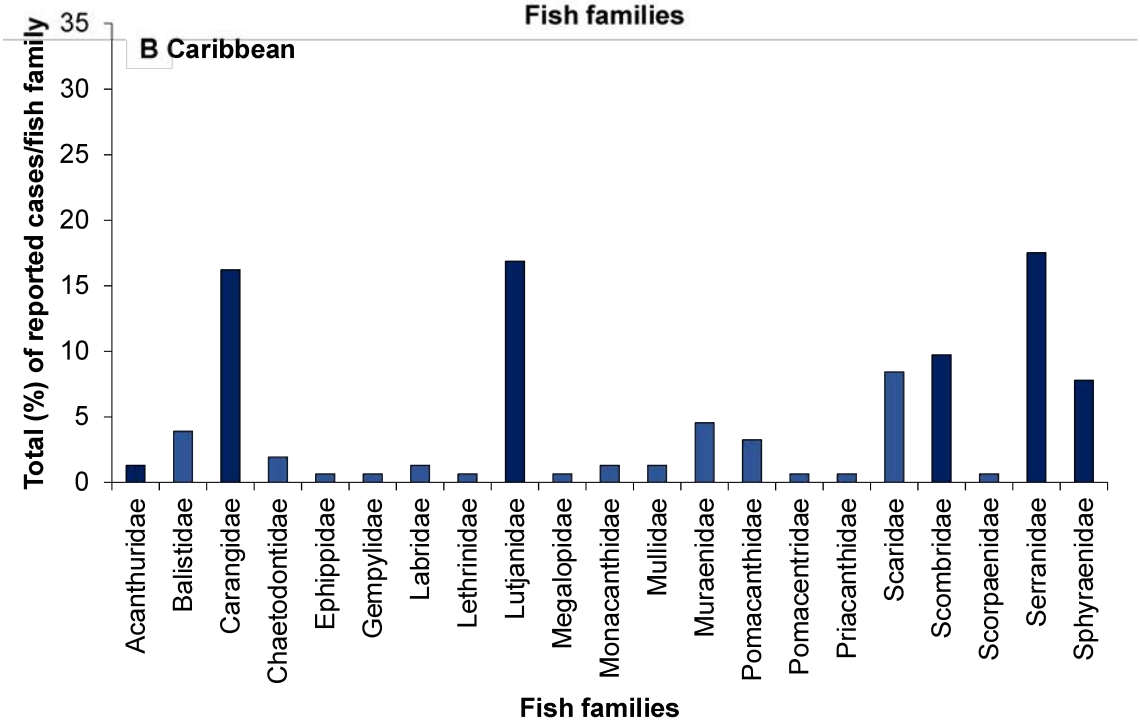
zone	# cases
1	28
2	35
3	6
4	12
5	5
	86

Appendix C

Chapter Two:

Supplementary Figure S2.1. Percent distribution of fish families associated with reported ciguatera in: (A) Pacific; and (B) Caribbean. The six fish families most frequently associated are shown in dark blue. Fish implicated with ciguatera from the Pacific and Caribbean was sourced from the Fishbase database (Froese & Pauly 2010).

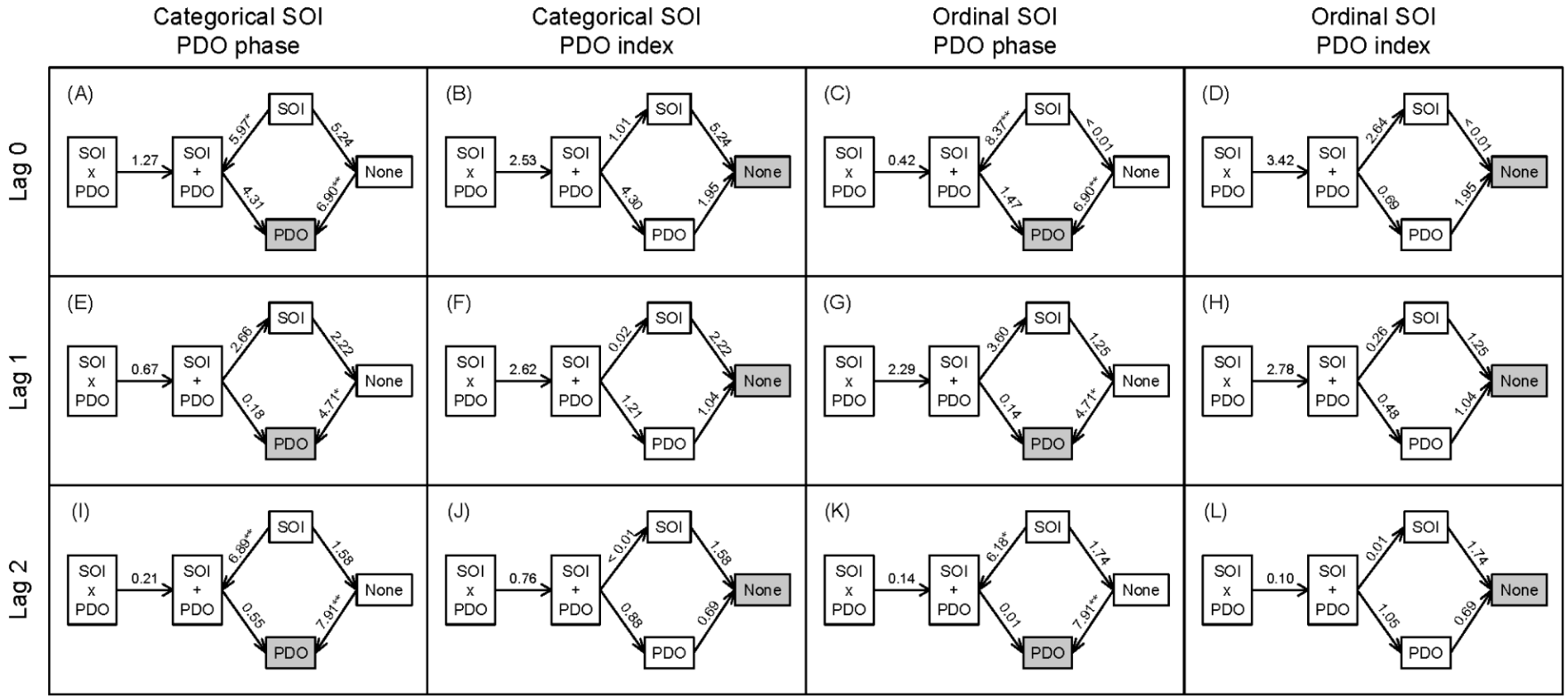

A Pacific
Total (%) of reported cases/fish family
35
30
25
20
15
10
5
0
Acanthuridae
Balistidae
Carangidae
Carcharhinidae
Gempylidae
Lethrinidae
Lutjanidae
Muraenidae
Nemipteridae
Scaridae
Scombridae
Serranidae
Sphyraenidae
Fish families


B Caribbean
Total (%) of reported cases/fish family
35
30
25
20
15
10
5
0
Acanthuridae
Balistidae
Carangidae
Chaetodontidae
Ephippidae
Gempylidae
Labridae
Lethrinidae
Lutjanidae
Megalopidae
Monacanthidae
Mullidae
Muraenidae
Pomacanthidae
Pomacentridae
Priacanthidae
Scaridae
Scombridae
Scorpaenidae
Serranidae
Sphyraenidae
Fish families

Appendix D

Chapter Three:

Supplementary Figure S3.1. Diagrams illustrating the results of model selection for the subsets of nested models. Within each panel, boxes labeled “SOI x PDO” include an interaction between the SOI and PDO explanatory variables, “SOI+PDO” are models including only additive effects of the two variables, “SOI” are models including only the SOI but not the PDO variable, “PDO” are models including the PDO but not the SOI variable, and “None” indicates a model where the number of cases is constant over time. Numbers on the arrows are likelihood ratio statistics for tests of the null hypothesis that the more complex model does not significantly improve the fit to the data. Where there are no asterisks, the likelihood ratio statistic is not significant, and thus the more complex model is rejected in favor of the simpler one (so the arrow points toward the simpler model). Asterisks indicate statistically significant support for the more complex model (*P<0.05; **P<0.01; ***P<0.001); in these cases, the arrow points towards the more complex model. In each panel, the best model in the subset is shaded in grey. Rows of panels correspond to models whose explanatory variables have different time lags (0, 1, or 2 years), and columns of panels correspond to different combinations of SOI and PDO variables (as described in the text, “Categorical” SOI refers to years classified as El Niño, La Niña, and normal; “Ordinal” SOI refers to yearly average numerical SOI value; “PDO phase” is either positive or negative; and “PDO Index” is the yearly average PDO index value).


Categorical SOI
PDO phase
Categorical SOI
PDO index
Ordinal SOI
PDO phase
Ordinal SOI
PDO index
Lag 0
Lag 1
Lag 2
(A)
(B)
(C)
(D)
(E)
(F)
(G)
(H)
(I)
(J)
(K)
(L)
SOI x PDO
SOI + PDO
SOI
PDO
None
1.27
5.97*
5.24
4.31
6.90**
2.53
1.01
4.30
1.95
0.42
8.37**
< 0.01
1.47
6.90**
3.42
2.64
< 0.01
0.69
1.95
0.67
2.66
2.22
0.18
4.71*
2.62
0.02
2.22
1.21
1.04
2.29
3.60
1.25
0.14
4.71*
2.78
0.26
1.25
0.48
1.04
0.21
6.89**
1.58
0.55
7.91**
0.76
< 0.01
1.58
0.88
0.69
0.14
6.18*
1.74
0.01
7.91**
0.10
0.01
1.74
1.05
0.69

Appendix E

Chapter Five:

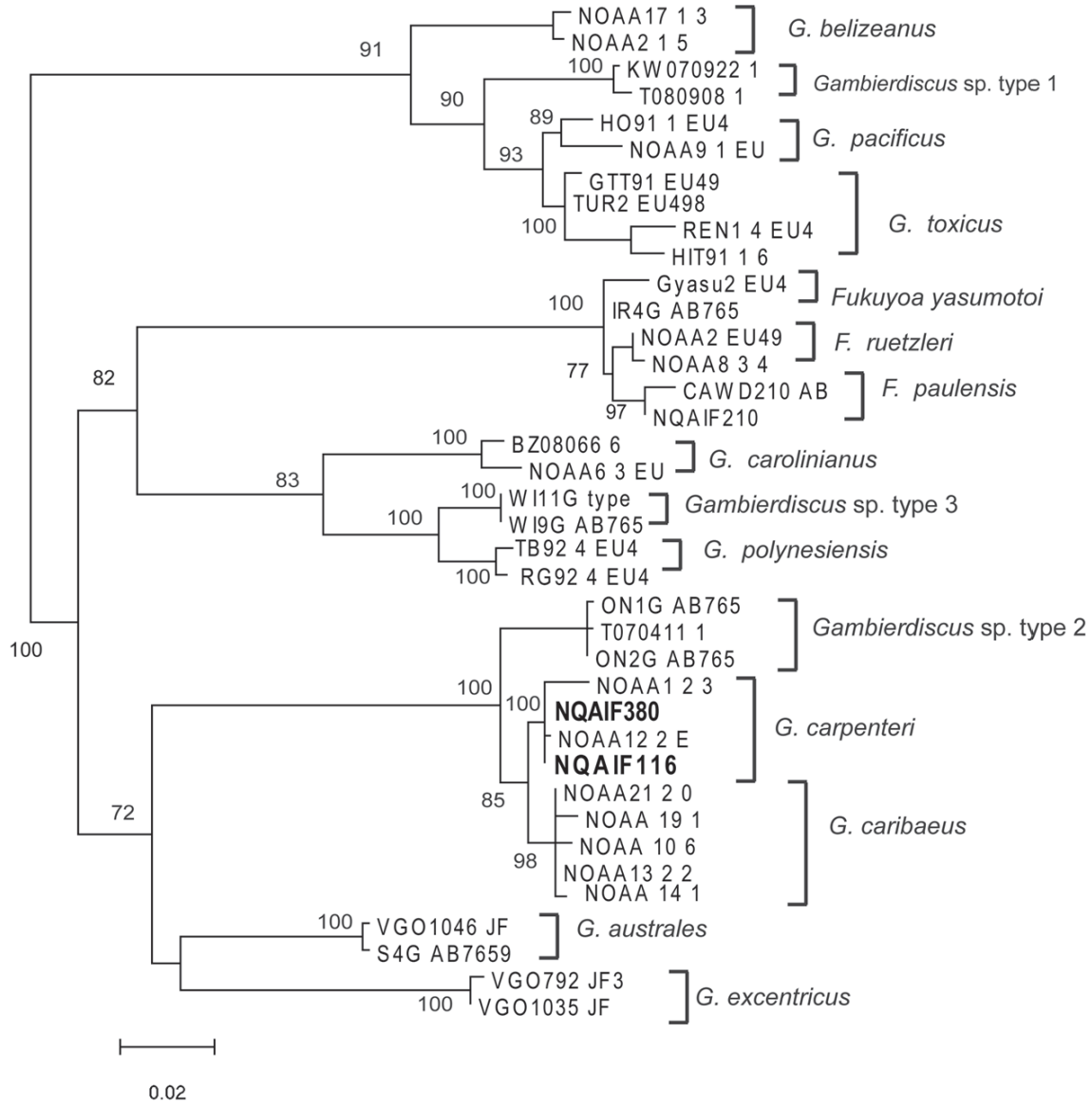
Supplementary Table S5.1. Statistical results of the two-way ANOVA testing for the effect of temperature and salinity on growth of *Gambierdiscus carpenteri* monocultures for NQAIF116 and NQAIF380 and mixed cultures of NQAIF116 with *Ostreopsis* sp. and *Prorocentrum lima*.

Monoalgal culture growth		**NQAIF116**			**NQAIF380**	
Treatment	**Df**	**F-value**	**P**	**Df**	**F-value**	**P**
Day	892	1548.353	**<0.0001**	888	749.824	**<0.0001**
Temperature	124	3.322	0.0708	124	50.611	**<0.0001**
Salinity	124	13.479	**0.0004**	124	40.522	**<0.0001**
Day:Temperature	892	11.716	**0.0006**	888	105.204	**<0.0001**
Day:Salinity	892	80.348	**<0.0001**	888	123.458	**<0.0001**
Temperature:Salinity	124	0.010	0.9206	124	2.577	0.1110
Day:Temperature:Salinity	892	0.852	0.3562	888	2.052	0.1524
NQAIF116 (culture growth in mixed assemblage)						
Day	918	410.707	**<0.0001**			
Gambierdiscus (mono:mixed)	126	0.368	0.5454			
Salinity	126	121.419	**<0.0001**			
Day:*Gambierdiscus*	918	1.740	0.1874			
Day:Salinity	918	243.923	**<0.0001**			
Gambierdiscus:Salinity	126	2.415	0.0935			
Day:*Gambierdiscus*:Salinity	918	7.168	**0.0008**			

Appendix F

Chapter Five:

Supplementary Figure S5.1. Maximum Likelihood phylogeny based on the D8-D10 region of the 28S rRNA gene illustrating the position of the strain (NQAIF380) within the genus *Gambierdiscus* (*sensu lato*). Branch labels represent bootstrap support based on 1000 pseudo-replicate datasets. Scale represent number of changes per nucleotide. The strain isolated in this study is shown in **bold as NQAIF380**.


NOAA17 1 3
NOAA2 1 5
G. belizeanus
KW070922 1
T080908 1
Gambierdiscus sp. type 1
HO91 1 EU4
NOAA9 1 EU
G. pacificus
GTT91 EU49
TUR2 EU498
REN1 4 EU4
HIT91 1 6
G. toxicus
Gyasu2 EU4
IR4G AB765
Fukuyoa yasumotoi
NOAA2 EU49
NOAA8 3 4
F. ruetzleri
CAWD210 AB
NQAIF210
F. paulensis
BZ08066 6
NOAA6 3 EU
G. carolinianus
WI11G type
WI9G AB765
Gambierdiscus sp. type 3
TB92 4 EU4
RG92 4 EU4
G. polynesiensis
ON1G AB765
T070411 1
ON2G AB765
Gambierdiscus sp. type 2
NOAA1 2 3
NQAIF380
NOAA12 2 E
NQAIF116
G. carpenteri
NOAA21 2 0
NOAA 19 1
NOAA 10 6
NOAA13 2 2
NOAA 14 1
G. caribaeus
VGO1046 JF
S4G AB7659
G. australes
VGO792 JF3
VGO1035 JF
G. excentricus
91
90
89
93
100
100
100
77
97
82
100
83
100
100
100
100
100
100
85
98
72
100
100
0.02

Appendix G

Chapter Six:

Supplementary table S6.1. Tukey post hoc test on relative population growth rate [r] for benthic dinoflagellates, *Gambierdiscus carpenteri, Prorocentrum lima* and *Ostreopsis* sp. at 24 and 28 °C and salinities of 26 and 36.

Supplementary table S6.1. Tukey pos doc test on relative population growth rate [r] for benthic dinoflagellates, *Gambierdiscus carpenteri, Prorocentrum lima* and *Ostreopsis* sp. at 24 and 28 °C and salinities of 26 and 36.

Tukey HSD test; variable Population growth rate (Population growth.sta)
Approximate Probabilities for Post Hoc Tests
Error: Between MS = .00010, df = 24.000

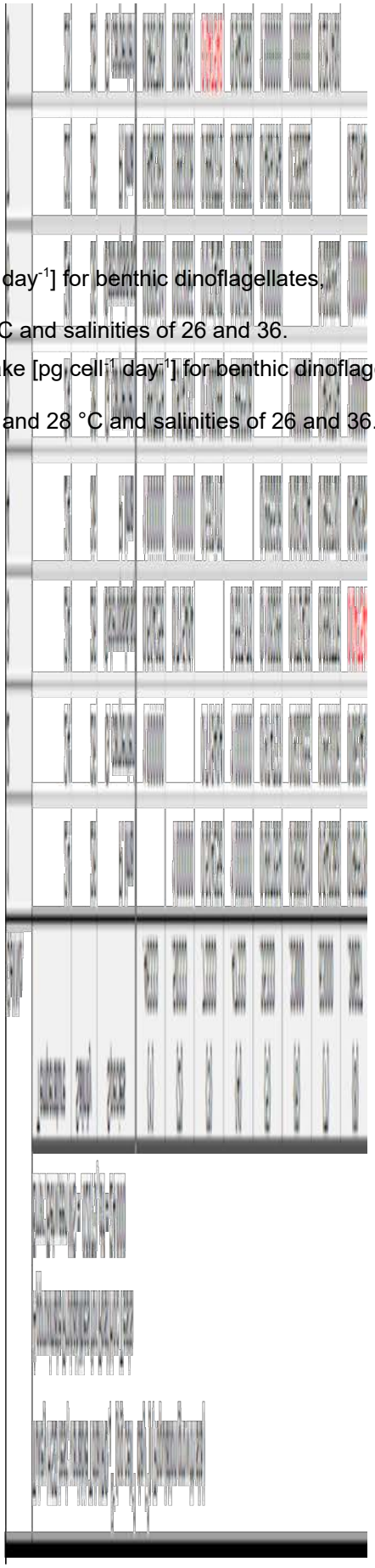
Cell No.	Temperature	Salinity	Species	{1} .20667	{2} .18667	{3} .18667	{4} .20333	{5} .22000	{6} .18667	{7} .18333	{8} .17333	{9} .22333	{10} .19333	{11} .22000	{12} .20333
1	24	26	P. lima		0.432531	0.432531	0.999999	0.888820	0.432531	0.233148	0.006678	0.681164	0.888820	0.888820	0.999999
2	24	26	G. carpenteri	0.432531		1.000000	0.681164	0.019678	1.000000	0.999999	0.888820	0.007781	0.999446	0.019678	0.681164
3	24	26	Osteropsis sp	0.432531	1.000000		0.681164	0.019678	1.000000	0.999999	0.888820	0.007781	0.999446	0.019678	0.681164
4	24	36	P. lima	0.999999	0.681164	0.681164		0.681164	0.681164	0.432531	0.048100	0.432531	0.983043	0.681164	1.000000
5	24	36	G. carpenteri	0.888820	0.019678	0.019678	0.681164		0.019678	0.007781	0.000517	0.999999	0.110791	1.000000	0.681164
6	24	36	Osteropsis sp	0.432531	1.000000	1.000000	0.681164	0.019678		0.999999	0.888820	0.007781	0.999446	0.019678	0.681164
7	28	26	P. lima	0.233148	0.999999	0.999999	0.432531	0.007781	0.999999		0.983043	0.003019	0.983043	0.007781	0.432531
8	28	26	G. carpenteri	0.019678	0.888820	0.888820	0.048100	0.000517	0.888820	0.983043		0.000272	0.432531	0.000517	0.048100
9	28	26	Osteropsis sp	0.681164	0.007781	0.007781	0.432531	0.999999	0.007781	0.003019	0.000272		0.048100	0.999999	0.432531
10	28	36	P. lima	0.888820	0.999446	0.999446	0.983043	0.110791	0.999446	0.983043	0.432531	0.048100		0.110791	0.983043
11	28	36	G. carpenteri	0.888820	0.019678	0.019678	0.681164	1.000000	0.019678	0.007781	0.000517	0.999999	0.110791		0.681164
12	28	36	Osteropsis sp	0.999999	0.681164	0.681164	1.000000	0.681164	0.681164	0.432531	0.048100	0.432531	0.983043	0.681164	

Appendix H

Chapter Six:

Supplementary Table S6.2. Tukey post hoc test on total nitrogen uptale [pg cell^{-1} day^{-1}] for benthic dinoflagellates, *Gambierdiscus carpenteri*, *Prorocentrum lima* and *Ostreopsis* sp. at 24 and 28 °C and salinities of 26 and 36.

Supplementary Table S6.3. Tukey post hoc test on total phosphate uptake [pg cell^{-1} day^{-1}] for benthic dinoflagellates, *Gambierdiscus carpenteri*, *Prorocentrum lima* and *Ostreopsis* sp. at 24 and 28 °C and salinities of 26 and 36.



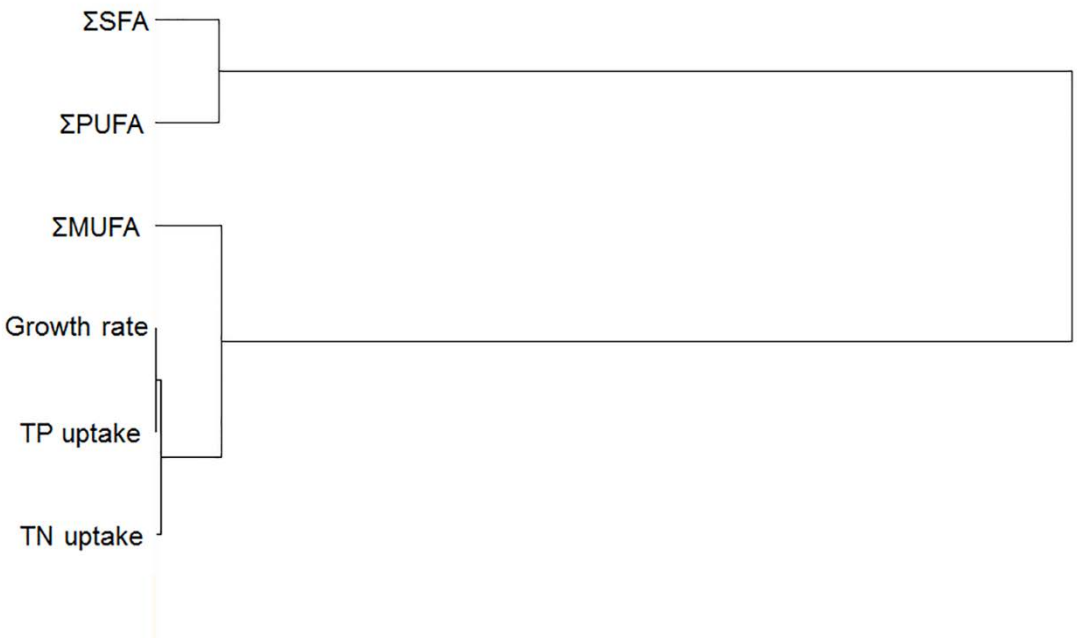
Supplementary Table S6.2. Tukey pos doc test on total nitrogen uptale [pg cell^{-1} day^{-1}] for benthic dinoflagellates, *Gambierdiscus carpenteri*, *Prorocentrum lima* and *Ostreopsis* sp. at 24 and 28 °C and salinities of 26 and 36.

Supplementary Table S6.3. Tukey pos doc test on total phosphate uptake [pg cell^{-1} day^{-1}] for benthic dinoflagellates, *Gambierdiscus carpenteri*, *Prorocentrum lima* and *Ostreopsis* sp. at 24 and 28 °C and salinities of 26 and 36.

Appendix I

Chapter Six:

Supplementary Figure S6.1. A Ward's hierarchical cluster analysis reveals two groups in the physiological response of benthic dinoflagellates to temperatures (24 and 28 °C) and salinities (36 and 26). One group contains Saturated Fatty Acids (SFA) and Polyunsaturated Fatty Acids (PUFA), the other group contains the Monounsaturated Fatty Acids (MUFA) with population growth rate, total phosphate (TP) and total nitrogen (TN) uptake.


ΣSFA
ΣPUFA
ΣMUFA
Growth rate
TP uptake
TN uptake

Appendix J

Chapter Six:

Supplementary Table S6.4. Average daily total nitrogen and phosphorus loads in $\mu g.L^{-1}$ for lagoon catchments in tropical (GBR) and temperate (New South Wales (NSW) and the northern Adriatic Sea) locations.

Location	Nitrogen	Phosphorus
Great Barrier Reef, Qld[1]	0.00137	0.0145
Merimbula, NSW[2]	0.77	0.101
Wallis Lake, NSW[2]	32	9.36
Northern Adriatic Sea, Mediterranean[3]	680	64
Caribbean	7	13.95

1(Furnas et al., 2005); 2(Scanes et al., 2007); 3(Accoroni et al., 2017)

Appendix K

Chapter Six:

Supplementary Table S6.5. Nutrient uptake rate ($pg.cell^{-1}.day^{-1}$) calculated from reported nutrient concentration [µmol] changes and population growth.

Location	Dinoflagellate	Estimated cells L^{-1}	pg N $cell^{-1}$ day^{-1}	Estimated cells L^{-1}	pg P $cell^{-1}$ day^{-1}	Salinity [g L^{-1}]	[°C]
Caribbean[1]	*G. toxicus*	20,408	343	25,272	552	35	27
GBR[2]	*G. carpenteri*	329	4.16	10,454	0.38	36	28
GBR[2]	*P. lima*	233	5.87	8,636	0.46	26	24
Mediterranean[3]	*P. lima*	12,551,180	54.2	8,078,239	7.97	25	20
GBR[2]	*Ostreopsis*	464	2.95	12,038	0.33	36	24
Mediterranean[4]	*O. ovata*	52,693,569	12.91	38,323,549	1.68	36	20

1(Lartigue et al., 2009); 2current study; 3(Vanucci et al., 2010); 4(Vanucci et al., 2012)